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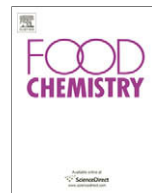

Evaluation of antioxidant ability of ethanolic extract from dill (*Anethum graveolens* L.) flower

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ABSTRACT

Antioxidant activities of ethanolic extract from dill flower and its various fractions were evaluated with 2,2-diphenyl-1-picrylhydrazyl radical scavenging, Trolox equivalent antioxidant capacity, reducing power, chelating power, and β-carotene bleaching assays. The flower extract was successively separated into *n*-hexane, ethyl acetate and ethanol soluble fractions by liquid–liquid partition. Dill leaf and seed extracts were used for comparison. In all assays, the flower extract showed higher antioxidant activity than the leaf and seed extracts. With regard to various fractions of the flower extract, the sequence for antioxidant activity was ethyl acetate fraction > ethanol fraction > original flower extract > *n*-hexane fraction. Phenols including flavonoids and proanthocyanidins should be responsible for antioxidant abilities of the flower extract. Chlorogenic acid, myricetin, and 3,3′,4′,5,7-pentahydoxyflavan (4 → 8)-3,3′,4′,5,7-pentahydoxyflavan were the major phenolic acid, flavonoid, and proanthocyanidin, respectively, in the dill flower extract.

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1. Introduction

Dill (*Anethum graveolens*) is an annual or biennial herb. It grows up to 90–120 cm tall and has slender branched stems, finely divided leaves, small umbels (2–9 cm diameter) of yellow flowers, and long spindle-shaped roots. In general, dill leaves (dill weeds) and seeds (small fragrant fruits) are used as seasoning. The leaves could be used in eggs, meats, salads, seafoods and soups; the seeds could be used in bread, and flavouring pickles and soups. Dill essential oil, extracted from both leaves and seeds, could also be used in chewing gums, candies and pickles (Just, 2008; Tucker, 2008; Zohary & Hopf, 2000).

The plant is native in Southwest Asia and is cultivated in Europe, India and the United States (Tucker, 2008). It is also successfully cultivated in Taiwan. Literature demonstrates that dill leaf consumption could lower the risk of cancer (Yang, Huang, Peng, & Li, 1996) and reduce the level of cholesterolaemia (Lanky, Schilcher, Phillipson, & Loew, 1993). Moreover, dill leaf, seed and their essential oil could provide good antioxidant activities (Delaquis, Stanich, Girard, & Mazza, 2002; Kmiecik, Gębezyński, & Jaworska, 2001; Mohammad Al-Ismail & Aburjai, 2004; Singh, Maurya, Lampasona, & de Catalan, 2005). Many reports indicate that plant flowers have remarkable antioxidant activity (Elzaawely, Xuan, Koyama, & Tawata, 2007; Ho, Hwang, Shen, & Lin, 2007; Kaur, Alam, Jabbar, Javed, & Athar, 2006; Susanti et al., 2007). There is, however, no thorough report on the antioxidant capacity of dill flower.

The antioxidant properties of ethanolic extract from the flower of dill cultivated in Taiwan and its various fractions (*n*-hexane, ethyl acetate and ethanol) were evaluated for 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging, Trolox equivalent antioxidant capacity (TEAC), reducing power, and β-carotene bleaching efficacies. Ethanolic extracts of dill leaf and seed were also used for comparison in all assays. Antioxidant components in these extracts were also determined.

2. Materials and methods

2.1. Samples

Fresh dill flowers, leaves and seeds (fruits) were obtained from Tainan District Agricultural Research and Extension Station (Tainan, Taiwan). All samples were lyophilised (at −50 °C for 48 h) in a freeze-drying system (Vastech Scientific Co., Ltd., Taipei, Taiwan) prior to extraction.

2.2. Chemicals

Ethanol (95%) (EtOH), methanol (MeOH), *n*-hexane (HX), ethyl acetate (EA), acetic acid (CH_3COOH), and formic acid (HCOOH)

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were purchased from Merck Co. (Darmstadt, Germany). Distilled deionized water (dd H_2O) was prepared by Ultrapure™ water purification system (Lotun Co., Ltd., Taipei, Taiwan). Ascorbic acid, aluminium chloride, 2-2′-azino-bis-(3-ethylbenz-thiazoline-6-sulphonic acid) (ABTS), ammonium formate ($HCOONH_4$), β-carotene, (+)-catechin, ethylenediaminetetraacetic acid (EDTA), Folin–Ciocalteu's phenol reagent, ferrozine, gallic acid, gentisic acid, chlorogenic acid, caffeic acid, (−)-epicatechin, *p*-coumaric acid, sinapic acid, benzoic acid, *p*-anisic acid, myricetin, quercetin, luteolin, kaempferol, apigenin, 6-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid (Trolox), linoleic acid, α-, β-, γ- and δ-tocopherols, vanillin, 2,2-diphenyl-1-picrylhydrazyl (DPPH), horseradish peroxidase, and trichloroacetic acid (TCA) were purchased from Sigma Co. (St. Louis, MO, USA). Hydrochloric acid (HCl), sodium carbonate (Na_2CO_3), sodium hydroxide (NaOH), sodium nitrite ($NaNO_2$), disodium hydrogen phosphate (Na_2HPO_4) and hydrogen peroxide (H_2O_2) were obtained from Wako Co. (Osaka, Japan). Ferrous chloride ($FeCl_3$), potassium ferricyanide ($K_3Fe(CN)_6$), and sodium dihydrogen phosphate (NaH_2PO_4) were purchased from Merck Co. (Darmstadt, Germany).

2.3. Preparation of dill extracts

For sample preparation, 50 g of each dried dill sample were extracted twice with 0.5 L of 95% ethanol at 25 °C for 24 h. The extracts were filtrated through Whatman No. 1 and combined followed by concentration using a rotary evaporator (Panchun Scientific Co., Kaohsiung, Taiwan) at 35 °C. The obtained dried extracts of dill flower, leaf and seed were 10.26, 9.85 and 6.83 g, respectively. In order to resolve the compounds contributing to antioxidant capacity of dill flower extract, the extract was further subjected to liquid–liquid partition successively with HX then EA to obtain the HX (4.57 g) and EA (4.14 g) soluble fractions. The remaining fraction was considered as the EtOH soluble fraction (1.42 g). The solvent of the fractions was also removed using a rotary evaporator after partition.

2.4. Antioxidant assays

Each sample was dissolved in 95% EtOH at a concentration 1 mg/mL and then diluted to prepare the series concentrations for antioxidant assays. Dill leaf and seed extracts were used for comparison in all assays.

2.4.1. DPPH radical scavenging activity assay

The assay was done according to the reports of Shimada, Fujikawa, Yahara, and Nakamura (1992) and Epsin, Soler-Rivas, and Wichers (2000). An aliquot of each sample (200 μL) was mixed with 50 μL of 1 mM DPPH (prepared with MeOH) followed by incubation for 30 min. The absorbance (Abs) was read at 517 nm (Multiskan Spectrum, Thermo Co., Vantaa, Finland). The scavenging activity was estimated based on the percentage of DPPH radical scavenged as the following equation: Scavenging effect (%) = $[Abs_{control} - (Abs_{sample} - Abs_{sample\ background})]/Abs_{control} \times 100$. EC_{50} value is the effective concentration that could scavenge 50% of the DPPH radicals. Ascorbic acid and (+)-catechin standards were used as positive references.

2.4.2. Trolox equivalent antioxidant capacity (TEAC) assay

The assay was carried out according to the methods of Arnao, Casas, Del Río, Acosta, and García-Cánovas. (1990) and Scalzo, Politi, Pellegrini, Mezzetti, and Battino (2005). The $ABTS^+$ solution ($OD_{734} = 0.70 \pm 0.03$) was prepared by mixing ABTS, H_2O_2 and peroxidase with the final concentrations of 100 μM, 50 μM and 4.4 unit/mL, respectively. An aliquot of each sample (30 μL) was mixed with 300 μL of the $ABTS^+$ solution for 3 min and the absorbance was determined at 734 nm. The scavenging percentage of $ABTS^+$ was calculated relative to Trolox. The Trolox calibration equation was $y = -0.729x + 0.7276$ (correlation coefficient, $R^2 = 0.9991$; y is the value of the absorbance; x is the value of the solution concentration). The TEAC value was expressed as mmole Trolox equivalent (TE)/g extract. (+)-Catechin standard was used as a positive reference.

2.4.3. Reducing power

Reducing power was determined as the method of Oyaizu (1986). An aliquot of each sample (125 μL) was mixed with 125 μL of sodium phosphate buffer (0.2 M, pH 6.6) and 125 μL of 1% $K_3Fe(CN)_6$ followed by incubation at 50 °C for 20 min. After adding 125 μL of 10% trichloroacetic acid, the mixture was centrifuged at 3750 g for 10 min (Hermle Z300K centrifuge, Hermle Labortechnik GmbH, Wehingen Württ, Germany). The supernatant solution (100 μL) was mixed with 100 μL of dd H_2O and 20 μL of 1% ferric chloride to react for 10 min. Subsequently, the absorbance was measured at 700 nm. The EC_{50} value is the concentration of sample at which the absorbance is 0.5. L-Ascorbic acid and (+)-catechin standards were used as positive references.

2.4.4. Chelating power

The ability of the extract to chelate iron (II) was estimated according to the method of Dastmalchi et al. (2008). An aliquot of each sample (200 μL) was mixed with 100 μL of $FeCl_2 \cdot 2H_2O$ (2.0 mmol/L) and 900 μL of MeOH. After 5 min incubation, the reaction was initiated by the addition of 400 mL of ferrozine (5.0 mmol/L). After 10 min incubation, the absorbance at 562 nm was recorded. The chelating activity (%) was calculated as the following equation: chelating activity (%) = $[Abs_{control} - (Abs_{sample} - Abs_{sample\ background})]/Abs_{control} \times 100$. EC_{50} value is the effective concentration that could chelate 50% of iron (II). EDTA and ascorbic acid were used as controls.

2.4.5. β-Carotene bleaching assay

The assay was performed as given by Elzaawely et al. (2007) and modified slightly. First, 2 mg of β-carotene dissolved in 10 ml of chloroform was mixed with 20 mg of linoleic acid and 200 mg of Tween80 followed by chloroform removing under nitrogen and 50 ml of distilled water adding with vigorous shacking to prepare β-carotene linoleate emulsion. An aliquot of each sample (30 μL) was mixed with 250 μL of the emulsion, and then the absorbance was determined at 470 nm at 45 °C for 2 h. β-Carotene bleaching inhibition was estimated as the following equation: Bleaching inhibition (%) = (β-carotene content after 2 h of assay/initial β-carotene content) × 100. EC_{50} value is the sample concentration that could give 50% antioxidant ability. (+)-Catechin standard was used as a positive reference.

2.5. Determination of phytochemicals

The amounts of total phenols were determined by a method with Folin–Ciocalteu's phenol reagent using gallic acid as a standard (Julkunen-Titto, 1985) and expressed as mg gallic acid equivalent (GAE)/g of dried extract. Flavonoid contents were surveyed through a method with 10% $AlCl_3$ H_2O solution using (+)-catechin as a standard (Zhishen, Mengcheng, & Jianming, 1999) and expressed as mg catechin equivalent (CE)/g of dried extract. Total monomeric anthocyanins were determined by the differential pH method (Giusti & Wrolstad, 2001) and data were expressed as mg cyanidin-3-glucoside equivalents (cy-3-gluE)/g of dried extract, using $\varepsilon = 26900$. Proanthocyanidin contents were measured by spectrophotometer at 550 nm, on the basis of a colorimetric reaction with 10% $NH_4Fe(SO_4)_2$ after dissolution in hydrochloric acid (2 M) containing *n*-butanol, as that reported by Porter and Rossi

(1986). Ascorbic acid was measured according to the report of Klein and Perry (1982). The determination of tocopherols was carried out according to the method of Carprenter (1979).

2.6. Analyses of flavonoids, phenolic acids and proanthocyanidin in dill flower extract

The analyses of flavonoids and phenolic acids were performed according to the report of Chen, Zuo, and Deng (2001). The HPLC system consisted of a *PrimeLine*™ Gradient Model 500G HPLC pump system (Analytical Scientific Instruments, Inc., El Sobrante, CA, USA) and an S-3210 photodiode-array detector (PDA) (Schambeck SFD GmbH, Bad Honnef, Germany). The flavonoids and phenolic acids were detected at both 280 and 360 nm. UV spectra were also recorded from 220 to 450 nm at a rate of 1.00 spectrum/s. The column was an Eclipse XDR-C_{18} reversed-phase column (150×4.6 mm, 5 μm; Waters). A gradient solvent system consisting of solvent A (dd H_2O/CH_3COOH, 97:3, v/v) and solvent B (MeOH) was used: 100–90% A from 0 to 10 min, 90–30% A from 10 to 32 min, 30–0% A from 32 to 45 min. The flow rate was 1.0 ml/min.

Proanthocyanidins were confirmed by the method of Xu, Zhang, Chen, and Tu (2006). That was executed on an Agilent 1100 series LC/MS Trap SL MS with Trap Control 4.2 and Bruker Daltonics Data Analysis 2.2. The HPLC apparatus was HP1100, which equipped a quaternary pump and a UV–vis detector. The column was a Zorbax SB-C_{18} column (250×4.6 mm, 5.0 μm, Agilent Co.). A gradient solvent system consisting of solvent A ($HCOONH_4$, adjusting pH to 3.0 with HCOOH) and solvent B (dd H_2O/MeOH, 90:10, v/v) was employed: 90% A from 0 to 5 min, 90–30% A from 5 to 50 min, 30–0% A from 50 to 55 min, 0–90% A from 55 to 60 min. The flow rate was 0.8 ml/min. Nitrogen was used as the nebulizing and drying gas. Esi conditions were as follows: nitrogen pressure, 5.00 psi; drying gas, 3.0 L/min at 325 °C; ion spray voltage, 3500 V. Mass spectra were recorded from *m/z* 50 to 1000.

2.7. Statistical analysis

Determination of phytochemical contents and all antioxidant ability assays were executed in triplicate and the mean values were calculated. The data were subjected to analysis of variance (ANOVA) and Duncan's multiple range tests were used to assess differences between means. A significant difference was considered at a level of $p < 0.05$. The regression analysis between total phenolic content and TEAC or antioxidant activity EC_{50} values were carried out with Microsoft Excel XP software.

3. Results and discussion

3.1. Contents of antioxidant components

Phenolic compounds widely exist in plants are bioactive substances. It is well known that they are highly effective antioxidants (Shahidi & Naczk, 2004; Shahidi & Wanasundara, 1992; Tapiero, Tew, Nguyen Ba, & Mathe, 2002). Plant phenols comprise a great diversity of compounds, such as flavonoids (anthocyanins, flavanols, flavonols, flavones, amongst others), proanthocyanidins also known as condensed tannins (the oligomeric and polymeric flavan-3-ols) and so on (Shahidi & Naczk, 2004). Flavonoids have the basic skeleton of diphenylpropanes (C_6–C_3–C_6) with various oxidation level of the central pyran ring (Shahidi & Naczk, 2004); they could provide strong antioxidant activities associated with their capacity to scavenge free radical and terminate radical chain reactions (Bors, Heller, Michel, & Saran, 1990). The proanthocyanidins, a group of biologically active polyphenolic bioflavonoids, have beneficial effects in radical scavenging and other relevant redox active properties (Bagchi et al., 1997). Ascorbic acid and tocopherols are the important antioxidants (vitamins) in organisms to quench free radicals also widely existing in plants (Vinson, Al Kharrat, & Andreoli, 2005).

The extraction yields of these dill samples can be ranked as flower (20.52 g/100 g of dried sample) > leaf (19.70 g/100 g of dried sample) > seed (13.66 g/100 g of dried sample). The flower extract had higher total amounts of polyphenols, flavonoids, anthocyanins and proanthocyanidins than its corresponding leaf and seed extracts. However, the leaf and seed extracts had the highest total amounts of ascorbic acid and tocopherols, respectively (Table 1). Only α- and γ-tocopherols were determined in these extracts (8.21 mg of α-tocopherol and 13.34 mg of γ-tocopherol in flower extract; 12.65 mg of α-tocopherol and 9.02 mg of γ-tocopherol in leaf extract; 15.27 mg of α-tocopherol and 32.06 mg of γ-tocopherol in seed extract). For the component contents (mg) in per gram of dried extract, polyphenols, flavonoids, anthocyanins and proanthocyanidins in the flower extract, ascorbic acid in the leaf extract, and tocopherols in the seed extract exhibited the highest levels, respectively (Table 1).

After liquid–liquid partition of the flower extract, the sequence of extraction yields of fractions were in the decreasing order of HX (9.14 g/100 g of dried sample) > EA (8.28 g/100 g of dried sample) > EtOH soluble fractions (2.84 g/100 g of dried sample). The highest total amounts of polyphenols, flavonoids, anthocyanins and proanthocyanidins were found in the EA soluble fraction, whereas the highest total amounts of ascorbic acid and tocopherols were observed in the EtOH and HX soluble fractions, respectively. Ascorbic and tocopherols could not be measured in the HX and EtOH soluble fractions, respectively (Table 1). For the component contents (mg) in per gram of dried extract, polyphenols and proanthocyanidins in the EA soluble fraction, flavonoids, anthocyanins and ascorbic acid in EtOH soluble fraction, and tocopherols in HX soluble fraction presented the highest levels, respectively (Table 1).

Phenolic level in flower (56.7 mg/g of dried extract) of *Alpinia zerumbet* (Pers.) B.L. Burtt. & R.M. Sm was also higher than that in its seed (13.7 mg/g of dried extract) (Elzaawely et al., 2007). Ho et al. (2007) found high amounts of phenols, flavonoids and proanthocyanidins (476.8, 156.0 and 186.7 mg/g of dried extract), and low ascorbic acid (2.2 mg/g of dried extract) in ethanolic extract of longan (*Dimocarpus longan* Lour.) flowers. Marimuthum, Wu, Chang, and Chang (2008) demonstrated that EA soluble fraction of ethanolic extract from bark of *Chamaecyparis obtuse* var. *formosan* had higher phenolic content (102.86 mg/g of dried extract) followed by EtOH soluble fraction, original bark extract and HX soluble fraction (90.72, 50.86, 27.71 mg/g of dried extract).

The results presented here show that phenols, flavonoids, proanthocyanidins, ascorbic acid, anthocyanins and tocopherols existed in ethanolic extracts of dill flower, leaf and seed. The contents (mg/g of dried extract) of phenols, anthocyanins and proanthocyanidins in these extracts were in the order of flower > leaf > seed extracts; moreover, ascorbic acid contents (mg/g of dried extract) were in the order: leaf > flower > seed extracts, and tocopherol contents (mg/g of dried extract) were in the order: seed > leaf > flower extracts. In addition, phenol and proanthocyanidin contents (mg/g of dried extract) in the flower extract and its fractions were in the order of EA soluble fraction > EtOH soluble fraction > original extract > HX soluble fraction; flavonoid contents were in the order of EtOH soluble fraction > EA soluble fraction > original extract > HX soluble fraction; anthocyanin and ascorbic acid contents (mg/g of dried extract) were in the order of EtOH soluble fraction > original extract > EA soluble fraction > HX soluble fraction; tocopherol contents (mg/g of dried extract) were in the order: HX soluble fraction > original extract > EA soluble fraction > EtOH soluble fraction. EtOH is a

Table 1
Contents of antioxidant components in dill flower, leaf and seed extracts, and various soluble fractions of the dill flower extract.

Extract	Content (mg/g of dried extract)					
	Phenols	Flavonoids	Anthocyanins	Proanthocyanidins	Ascorbic acid	Tocopherol
Flower extract	144.85 ± 11.62 c (1486.16)	48.24 ± 3.05 c (494.94)	8.27 ± 0.41 b (84.85)	47.05 ± 2.45 c (482.73)	2.03 ± 0.25 c (20.83)	2.10 ± 0.13 d (21.55)
EA fraction of flower extract	196.65 ± 13.62 a (814.13)	63.13 ± 2.57 b (261.34)	7.97 ± 0.68 b (32.98)	69.00 ± 4.01 a (285.66)	1.41 ± 0.31 d (5.83)	0.98 ± 0.07 e (4.09)
EtOH fraction of flower extract	173.52 ± 9.83 b (246.40)	67.10 ± 3.43 a (95.28)	19.53 ± 0.38 a (27.74)	56.85 ± 2.69 b (80.73)	8.96 ± 0.67 a (12.72)	ND
HX fraction of flower extract	78.80 ± 4.66 f (360.32)	20.02 ± 1.18 f (91.51)	2.35 ± 0.14 d (10.74)	20.68 ± 3.18 f (94.53)	ND	3.17 ± 0.28 b (14.50)
Leaf extract	136.53 ± 8.93 d (1344.82)	37.21 ± 2.56 d (366.52)	5.12 ± 0.34 c (50.43)	38.21 ± 1.93 d (376.37)	3.18 ± 0.22 b (31.32)	2.25 ± 0.15 c (21.67)
Seed extract	130.54 ± 12.51 e (891.59)	33.62 ± 1.76 e (229.62)	2.18 ± 0.11 d (14.89)	29.71 ± 1.60 e (202.92)	1.94 ± 0.10 c (15.30)	6.93 ± 0.34 a (47.33)

Values (mean ± SD, $n = 3$) in the same column followed by a different letter are significantly different ($p < 0.05$).
Values in parentheses are total amounts (mg) in extracts.
ND, not detected.

polar solvent whilst EA is a moderately polar solvent compared to the nonpolar solvent HX. Therefore, fat-soluble tocopherol was predominantly in the HX fraction and polar ascorbic acid was mainly partitioned into EtOH. Phenols, anthocyanins, proanthocyanidins and flavonoids all are different polar mixtures of compounds which result in appropriate fraction yields in EA and EtOH solvents.

3.2. Antioxidant activities of dill flower extract and its fractions

For TEAC assay Table 2, shows that EA soluble fraction of the ethanolic extract of dill flowerhad the highest antioxidant ability (TEAC value = 0.69 mmol TE/g of dried extract). Its EtOH soluble fraction (TEAC value = 0.62 mmol TE/g of dried extract) and the original dill flower extract (TEAC value = 0.46 mmol TE/g of dried extract) were the second and third. Their antioxidant abilities were higher than ethanolic extracts of dill leaf (TEAC value = 0.40 mmol TE/g of dried extract) and seed (TEAC value = 0.38 mmol TE/g of dried extract). HX soluble fraction of the dill flower extract presented the lowest antioxidant ability (TEAC value = 0.27 mmol TE/g of dried extract).

Fig. 1A shows that the DPPH radical scavenging ability of samples can be ranked as EA soluble fraction > EtOH soluble fraction > flower extract > leaf extract > seed extract > HX soluble fraction. The scavenging abilities on DPPH radicals at 0.1 mg of dried extract/mL were 55.84%, 48.89% and 45.42% for the flower, leaf and seed extracts, and 80.84%, 65.88% and 12.57% for the EA, EtOH and HX soluble fractions, respectively. At 0.4 mg/mL, the scavenging abilities could be increased to 86.01%, 82.50% and 82.30% for the flower, leaf and seed extracts, and 93.12%, 90.13% and 50.11% for the EA, EtOH and HX soluble fractions, respectively. The EC_{50} values of scavenging DPPH radicals for the flower, leaf and seed extracts were 85.29, 107.29 and 118.63 μg of dried extract/mL, respectively, and those for the EA, EtOH and HX soluble fractions were 28.15, 56.83 and 399.07 μg of dried extract/mL, respectively (Table 2).

Fig. 1B shows that reducing power increased with concentration of each sample. The sequence for reducing power was EA soluble fraction > EtOH soluble fraction > flower extract > leaf extract seed extract > HX soluble fraction. The EC_{50} values of reducing power for the flower, leaf and seed extracts were 306.25, 342.11 and 367.65 μg of dried extract/mL, respectively, and those for the EA, EtOH and HX soluble fractions were 28.15, 56.83 and 708.33 μg of dried extract/mL, respectively (Table 2).

Except HX soluble fraction, all extracts were better ferrous ion chelators compared to ascorbic acid (Fig. 1C). The chelating activity increased with concentration of each sample. The sequence for chelating power was EA soluble fraction EtOH soluble fraction > flower extract > leaf extract > seed extract > HX soluble fraction. However, all extracts presented much lower chelating power than EDTA. The EC_{50} values of chelating power for the flower, leaf and seed extracts were13791.05, 14536.97 and 14857.21 μg of dried extract/mL, respectively, and those for the EA, EtOH and HX soluble fractions were 12895.99, 12960.13 and 13791.05 μg of dried extract/mL, respectively (Table 2).

Table 2
TEAC value and EC_{50} values of scavenging ability on DPPH radicals, reducing power, chelating power and inhibition of β-carotene bleaching for dill leaf, seed and flower extracts, various soluble fractions of the dill flower extract.

Extract	TEAC (mmol TE[a]/g dried extract)	EC_{50}[b] (μg of dried extract/mL)			
		Scavenging ability on DPPH radicals	Reducing power	Chelating power	β-Carotene bleaching inhibition
Flower extract	0.46 ± 0.04 d	85.29 ± 6.11 d	306.25 ± 20.02 d	13791.05 ± 1021.21 c	156.08 ± 9.24 c
EA fraction of flower extract	0.69 ± 0.04 b	28.15 ± 0.22 f	204.2 ± 16.03 f	12895.99 ± 1089.45 d	72.64 ± 3.05 e
EtOH fraction of flower extract	0.62 ± 0.03 c	56.83 ± 0.35 e	227.17 ± 13.32 e	12960.13 ± 987.54 d	98.8 ± 6.17 d
n-Hexane fraction of flower extract	0.27 ± 0.01 f	399.07 ± 14.21 a	708.33 ± 35.78 a	>100,000	398.89 ± 23.27 a
Leaf extract	0.40 ± 0.02 e	107.29 ± 9.02 c	342.11 ± 18.02 c	14536.97 ± 978.43 b	176.79 ± 10.14 b
Seed extract	0.38 ± 0.03 e	118.63 ± 10.13 b	367.65 ± 15.45 b	14857.21 ± 1123.60 a	184.11 ± 12.37 b
Ascorbic acid	–	8.60 ± 0.52 g	17.09 ± 1.24 h	>100,000	–
(+)-Catechin	1.74 ± 0.09 a	7.44 ± 0.65 g	30.46 ± 2.01 g	–	31.06 ± 2.44 f
EDTA	–	–	–	33.19 ± 2.01	–

Values (mean ± SD, $n = 3$) in the same column followed by a different letter are significantly different ($p < 0.05$).
[a] TE, Trolox equivalent.
[b] EC_{50} means the effective concentration providing 50% antioxidant activity.

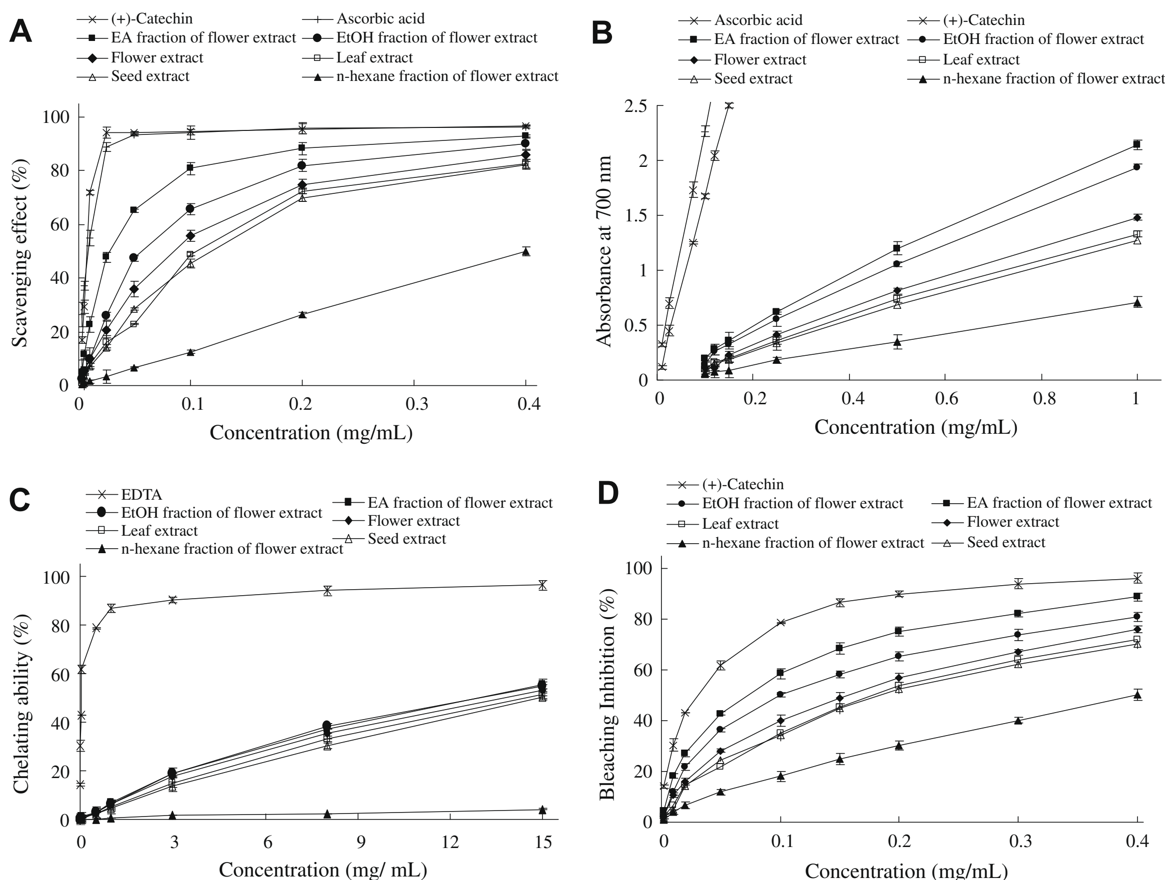

Fig. 1. Antioxidant activities of dill flower, leaf and seed extracts, and different soluble fractions of the dill flower extract: (A) scavenging DPPH radicals, (B) reducing power, (C) chelating power, and (D) β-carotene bleaching inhibition.

With regard to the β-carotene bleaching assay Fig. 1D, shows that the antioxidant activity of samples can be ranked as EA soluble fraction > EtOH soluble fraction > flower extract > leaf extract seed extract > HX soluble fraction. At 0.1 mg of dried extract/mL, β-carotene bleaching inhibitions were 40.04%, 35.33% and 34.03% for the flower, leaf and seed extracts, and 58.62%, 50.33% and 18.04% for the EA, EtOH and HX fractions, respectively. At 0.4 mg of dried extract/mL, the inhibition was increased to 76.12%, 72.23% and 70.11% for the flower, leaf and seed extracts, and 88.92%, 80.94%, and 50.09% for the EA, EtOH and HX soluble fractions, respectively. The EC_{50} values of β-carotene bleaching inhibition for the flower, leaf and seed extracts were 156.08, 176.79 and 184.11 μg of dried extract/mL, respectively, and those for the EA, EtOH and HX soluble fractions were 72.64, 98.80 and 398.89 μg of dried extract/mL, respectively (Table 2).

Through correlation analysis for phytochemical contents (mg/g of dried extract) with TEAC and antioxidant activity EC_{50} values for the dill flower extract and its various soluble fractions, the contents of phenols, flavonoids and proanthocyanidins presented high correlation with the TEAC and EC_{50} values of DPPH radical scavenging ability, reducing power and β-carotene blenching inhibition ($R^2 > 0.91$) (Table 3). Moreover, the contents of phenols, flavonoids and proanthocyanidins contents also exhibited good correlation with EC_{50} value of ferrous ion chelating power ($R^2 > 0.83$). Never-

Table 3
Correlations[a] established between each component content with TEAC/antioxidant activity EC_{50} values.

Assay	Equation		
	Phenols	Flavonoids	Proanthocyanidins
TEAC	$Y = 0.0036X - 0.0281$ $R^2 = 0.9817$	$Y = 0.0084X + 0.0913$ $R^2 = 0.9299$	$Y = 0.009X + 0.0758$ $R^2 = 0.9742$
EC_{50} of DPPH radical scavenging ability	$Y = -3.24X + 623.33$ $R^2 = 0.9166$	$Y = -7.7283X + 525.83$ $R^2 = 0.9115$	$Y = -7.7022X + 511.23$ $R^2 = 0.9145$
EC_{50} of reducing power	$Y = -4.4662X + 1024.5$ $R^2 = 0.9384$	$Y = -10.733X + 894.1$ $R^2 = 0.9473$	$Y = -10.991X + 893.42$ $R^2 = 0.9206$
EC_{50} of chelating power	$Y = -776.48X + 150184$ $R^2 = 0.8742$	$Y = -1693X + 116677$ $R^2 = 0.8923$	$Y = -1907.4X + 127221$ $R^2 = 0.8353$
EC_{50} of β-carotene blenching inhibition	$Y = -2.8621X + 606.5$ $R^2 = 0.9614$	$Y = -6.8192X + 519.99$ $R^2 = 0.9539$	$Y = -7.0562X + 523.09$ $R^2 = 0.9465$

Y is the TEAC or EC_{50} value; X is the total phytochemical content.
[a] Dill flower extract and its EA, EtOH and HX soluble fractions were used in the correlations.

Table 4
Flavonoids and phenolic acids in dill flower extract.

Compound	Retention time (min)[a]	Content (mg/g of dried extract)
Gallic acid	3.08	10.23 ± 0.54[b]
Gentisic acid	13.82	ND[c]
(+)-Catechin	14.92	ND
Chlorogenic acid	16.38	26.56 ± 1.13
Caffeic acid	18.01	ND
(−)-Epicatechin	19.16	10.13 ± 0.66
p-Coumaric acid	22.57	12.32 ± 1.02
Sinapic acid	25.24	ND
Benzoic acid	27.38	4.12 ± 0.26
p-Anisic acid	29.16	7.23 ± 0.48
Myricetin	30.55	11.27 ± 1.02
Quercetin	32.98	9.42 ± 0.82
Luteolin	37.94	4.41 ± 0.24
Kaempferol	43.67	4.52 ± 0.30
Apigenin	44.42	ND
Total phenolic acid		60.46
Total flavonoid		39.65
Total amount		100.11

[a] HPLC conditions are showed in Section 2.6.
[b] Values are mean values ± standard deviation ($n = 3$).
[c] ND, not detected.

Table 5
Proanthocyanidins in dill flower extract.

Chemical name	Molecular weight	Molecular ion [M−H]$^-$, (fragments)	Relative content (%)
3,3′,4′,5,7-Pentahydoxyflavan((−)-epicatechin)	290	289 (271, 245, 231, 205, 179, 151)	25.1
3′,4′,5,7-Tetrahydoxyflavan (4 → 8)-3,3′,4′,5,7-pentahydoxyflavan	562	561 (543, 435, 329, 289, 271, 245)	33.3
3,3′,4′,5,7-Pentahydoxyflavan (4 → 8)-3,3′,4′,5,7-pentahydoxyflavan	578	577 (559, 451, 425, 289, 245)	41.6

theless, correlations between contents of anthocyanins, ascorbic acid and tocopherols and these antioxidant activity values could not be established well.

Elzaawely et al. (2007) found that flower and seed of s *A. zerumbet* (Pers.) B.L. Burtt. & R.M. Sm. exhibited antioxidant actions. Although the flower had stronger capacity than the seed in the inhibition of β-carotene bleaching assay, no differences were observed between the flower and seed in their DPPH radical scavenging abilities. Our study showed that dill flower had significantly higher antioxidant ability than its leaf and seed in all assays (TEAC, scavenging DPPH radicals, reducing power and β-carotene bleaching inhibition), whereas dill leaf and seed presented similar antioxidant activities. Marimuthum et al. (2008) prepared EA, EtOH and HX soluble fractions from ethanolic extract of bark of *C. obtuse* var. *formosan*, and found that the sequence for DPPH radical scavenging ability and reducing power was in the order of EA soluble fraction > EtOH soluble fraction > original bark extract > HX soluble fraction; the sequence for β-carotene bleaching inhibition was EA fraction > original bark extract > EtOH soluble fraction > HX soluble fraction; the sequence for total antioxidant activity (TEAC assay) was EtOH soluble fraction > EA soluble fraction > original bark extract > HX soluble fraction. Our study also prepared EA, EtOH and HX soluble fractions from ethanolic extract of dill flower. In all tested methods, the EA soluble fraction showed the highest antioxidant activity and the EtOH soluble fraction next; moreover, the original flower extract had much higher effect than the HX soluble fraction.

Several studies showed a correlation between contents of polyphenols and flavonoids and antioxidant activities for herb (flower, fruit, leaf, seed and so on) extracts (Dastmalchi et al., 2008; Elzaawely et al., 2007; Mohammad Al-Ismail & Aburjai, 2004; Škerget, Kotnik, Hadolin, Hraš, & Simonic, 2005). Škerget et al. (2005) indicated that phenols, proanthocyanidins, flavones and flavonols in St. John's wort (*Hypericum perforatum*) and oregano (*Origanum vulgare*) markedly contributed to their antioxidant activities. Our results illustrated that phenols including flavonoids and proanthocyanidins should be responsible for the effective antioxidant properties of the dill flower extract.

3.3. Confirmation of flavonoids, phenolic acids and proanthocyanidins in dill flower extract

In order to know what flavonoids, phenolic acids and proanthocyanidins are present in the dill flower extract, the extract was analysed by HPLC and LC-MS further. Flavonoids including (−)-epicatechin, myricetin, quercetin, luteolin and kaempferol, and phenolic acids including gallic acid, chlorogenic acid, *p*-coumaric acid, benzoic acid and *p*-anisic acid could be found in the extract (Table 4). The contents of phenolic acids and flavonoids were in the range of 4.12–26.56 and 4.41–11.27 mg/g of dried extract, respectively. Proanthocyanidins in the extract could be identified as 3,3′,4′,5,7-pentahydoxyflavan ((−)-epicatechin), 3′,4′,5,7-tetrahydoxyflavan (4 → 8)-3,3′,4′,5,7-pentahydoxyflavan, and 3,3′,4′,5,7-pentahydoxyflavan (4 → 8)-3,3′,4′,5,7-pentahydoxyflavan. Their mass spectra corresponded to those reported by Xu et al. (2006) and Hsieh, Shen, Kuo, and Hwang (2008) (Table 5). Chlorogenic acid (26.56 mg/g of dried extract), myricetin (11.27 mg/g of dried extract), and 3,3′,4′,5,7-pentahydoxyflavan (4 → 8)-3,3′,4′,5,7-pentahydoxyflavan (16.79 mg/g of dried extract) were the highest levels of phenolic acid, flavonoid, and proanthocyanidin, respectively, in the dill flower extract (Tables 4 and 5).

4. Conclusions

Ethanolic extract of dill flower had higher antioxidant activity than corresponding extracts of dill leaf and seed. Through correlation analysis of phytochemical contents and antioxidant capacities for the original dill flower extract and its HX, EA and EtOH soluble fractions, the representative antioxidant components could be regarded as phenols, flavonoids and proanthocyanidins. Chlorogenic acid, myricetin, and 3,3′,4′,5,7-pentahydoxyflavan (4 → 8)-3,3′,4′,5,7-pentahydoxyflavan were the major phenolic acid, flavonoid, and proanthocyanidin, respectively, in the dill flower extract. The results could provide additional information for dill antioxidant property.

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References

Arnao, M. B., Casas, J. L., Del Río, J. A., Acosta, M., & Cánovas, García (1990). An enzymatic colorimetric method for measuring naringin using 2,2′-azino-bis-(3-ethylbenzthiazoline-6-sulphonic acid)(ABTS) in the presence of peroxidase. *Analytical Biochemistry, 185*, 335–338.

Bagchi, D., Garg, A., Krohn, R. L., Bagchi, M., Tran, M. X., & Stohs, S. J. (1997). Oxygen free radical scavenging abilities of vitamins C and E, and a grape seed proanthocyanidin extract in vitro. *Research Communications in Molecular Pathology and Pharmacology, 95*, 179–189.

Bors, W., Heller, W., Michel, C., & Saran, M. (1990). Flavonoids as antioxidants: Determination of radical scavenging efficiencies. *Methods in Enzymology, 186*, 67–103.

Carprenter, A. P. (1979). Determination of tocopherols in vegetable oils. *Journal of the American Oil Chemists' Society, 56*, 668–672.

Chen, Hao, Zuo, Y., & Deng, Y. (2001). Separation and determination of flavonoids and other phenolic compounds in cranberry juice by high-performance liquid chromatography. *Journal of Chromatography A, 913*, 387–395.

Dastmalchi, K. D., Doeman, H. J. D., Oinonen, P. P., Darwis, Y., Laaso, I., & Hiltunen, R. (2008). Chemical composition and in vitro antioxidative activity of a lemon balm (*Melissa officinalis* L.) extract. *LWT, 41*, 391–400.

Delaquis, P. J., Stanich, K., Girard, B., & Mazza, G. (2002). Antimicrobial activity of individual and mixed fractions of dill, cilantro, coriander and eucalyptus essential oils. *International Journal of Food Microbiology, 74*, 101–109.

Elzaawely, A. A., Xuan, T. D., Koyama, H., & Tawata, S. (2007). Antioxidant activity and contents of essential oil and phenolic compounds in flowers and seeds of *A. zerumbet* (Pers.) B.L. Burtt. & R.M. Sm.. *Food Chemistry, 104*, 1648–1653.

Epsin, J. C., Soler-Rivas, C., & Wichers, H. J. (2000). Characterization of the total free radical scavenger capacity of vegetable oils and oil fractions using 2,2-diphenyl-2-picrylhydrazyl radical. *Journal of Agricultural and Food Chemistry, 48*, 648–656.

Giusti, M. M., & Wrolstad, R. E. (2001). Anthocyanins. Characterization and measurement of anthocyanins by UV–visible spectroscopy. In *Current protocols in food analytical chemistry, unit F1.2.1–13*. New York: John Wiley & Sons.

Ho, S., Hwang, L. S., Shen, Y., & Lin, C. (2007). Suppressive effect of a proanthocyanidin-rich extract from longan (*D. longan* Lour.) flowers on nitric oxide production in LPS-stimulated macrophage cells. *Journal of Agricultural and Food Chemistry, 55*, 10664–10670.

Hsieh, M. C., Shen, Y. J., Kuo, Y. H., & Hwang, L. S. (2008). Antioxidative activity and active components of Longan (*D. longan* Lour.) flower extracts. *Journal of Agricultural and Food Chemistry, 56*, 7101–7106.

Julkunen-Titto, R. (1985). Phenolic constituents in the leaves of northern willows: Methods for the analysis of certain phenolics. *Journal of Agricultural and Food Chemistry, 33*, 213–217.

Just, T. "Dill" (2008). *Encyclopedia Americana*. Grolier online, 30 June 2008. <http://ea.grolier.com/cgi-bin/article?assetid=0127540–00>.

Kaur, G., Alam, M. S., Jabbar, Z., Javed, K., & Athar, M. (2006). Evaluation of antioxidant activity of *Cassia siamea* flowers. *Journal of Ethnopharmacology, 108*, 340–348.

Klein, B. P., & Perry, A. K. (1982). Ascorbic acid and vitamin A activity in selected vegetables from different geographical areas of United State. *Journal of Food Science, 47*, 941–945.

Kmiecik, W., Gębezyński, P., & Jaworska, G. (2001). Effect of the cultivar, usable part and growing period on the content of selected antioxidative components in dill (*A. graveolens* L.) Acta Agria et Silvestria s. *Agraria, 39*, 35–48.

Lanky, P. S., Schilcher, H., Phillipson, J. D., & Loew, D. (1993). Plants that lower cholesterol. *Acta Horticulturae, 332*, 131–136.

Marimuthum, P., Wu, C. L., Chang, H. T., & Chang, S. T. (2008). Antioxidant activity of ethanolic extract from bark of *C. obtuse* var. *formosan*. *Journal of the Science of Food and Agriculture, 88*, 1400–1405.

Mohammad Al-Ismail, K., & Aburjai, T. (2004). Antioxidant activity of water and alcohol extracts of chamomile flowers, anise seeds and dill seeds. *Journal of the Science of Food and Agriculture, 84*, 173–178.

Oyaizu, M. (1986). Antioxidant activity of browning products of glucosamine fractionated by organic solvent and thin-layer chromatography. *Nippon Shokulin Kogyo Gakkaishi, 35*, 771–775.

Porter, L. J., & Rossi, J. A. (1986). The conversion of procyanidins and prodelphinidins to cyanidins and delphinidins. *Phytochemistry, 25*, 225–230.

Scalzo, J., Politi, A., Pellegrini, N., Mezzetti, B., & Battino, M. (2005). Plant genotype affects total antioxidant capacity and phenolic contents in fruit. *Nutrition, 21*, 207–213.

Shahidi, F., & Naczk, M. (2004). *Phenolics in food and nutraceuticals*. Boca Raton, FL: CRC Press. pp. 403, 421–426.

Shahidi, F., & Wanasundara, P. K. J. P. D. (1992). Phenolic antioxidants. *CRC Critical Reviews in Food Science and Nutrition, 32*, 67–103.

Shimada, K., Fujikawa, K., Yahara, K., & Nakamura, T. (1992). Antioxidative properties of Xanthan on the autoxidation of soybean oil in cyclodextrin emulsion. *Journal of Agricultural and Food Chemistry, 40*, 945–948.

Singh, G., Maurya, S., Lampasona, M. P., & de Catalan, C. (2005). Chemical constituents, antimicrobial investigations, and antioxidative potentials of *A. graveolens* L. essential oil and acetone extract: Part 52. *Journal of Food Science, 70*, M208–M215.

Škerget, M., Kotnik, P., Hadolin, M., Hraš, A. R., & Simonic, M. (2005). Phenols, proanthocyanidins, flavones and flavonols in some plant materials and their antioxidant activities. *Food Chemistry, 89*, 191–198.

Susanti, D., Sirat, H. M., Ahmad, F., Ali, R. M., Aimi, N., & Kitajima, M. (2007). Antioxidant and cytotoxic flavonoids from the flowers of *Melastoma malabathricum* L.. *Food Chemistry, 103*, 710–716.

Tapiero, H., Tew, K. D., Nguyen Ba, G., & Mathe, G. (2002). Polyphenols: Do they play a role in the prevention of human pathologies? *Biomedicine and Pharmacotherapy, 56*, 200–207.

Tucker, A. O. "Dill" (2008). *Grolier multimedia encyclopedia*. Grolier online, 30 June 2008. <http://gme.grolier.com/cgi-bin/article?assetid=0085590–0>.

Vinson, J. A., Al Kharrat, H., & Andreoli, L. (2005). Effect of Aloe vera preparations on the human bioavailability of vitamins C and E. *Phtytomedicine, 12*, 760–765.

Xu, Y., Zhang, Y., Chen, M., & Tu, P. (2006). Fatty acids, tocopherols and proanthocyanidins in bramble seeds. *Food Chemistry, 99*, 586–590.

Yang, Y., Huang, C. Y., Peng, S. S., & Li, J. (1996). Carotenoid analysis of several dark-green leafy vegetables associated with a lower risk of cancers. *Biomedical and Environmental Sciences, 9*, 386–392.

Zhishen, J., Mengcheng, T., & Jianming, W. (1999). The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. *Food Chemistry, 64*, 555–559.

Zohary, D., & Hopf, M. (2000). *Domestication of plants in the Old World* (3rd ed.). Oxford: University Press. p. 206.