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In vitro antioxidant properties and total phenolic contents of wetland medicinal plants in Taiwan

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ABSTRACT. The aim of this study was to examine the possible antioxidant activities of the methanol and water extracts of 31 medicinal wetland plants in Taiwan. We assayed for such properties such as: TEAC, DPPH radical scavenging, total polyphenol content, total flavonoid and total flavonol contents using the reducing power method. Our results showed that *Rotala rotundifolia*, *Juncus effusus* var. *decipiens*, *Cyperus iria*, *Salix warburgii*, *Lindernia antipoda*, *Kyllinga brevifolia*, and *Typha orientalis* possessed both high antioxidant activities and high total polyphenol contents. There was a low correlation between TEAC and total polyphenol content (water extracts, R^2=0.14; methanol extracts, R^2=0.23) thus eliminating high phenolic content as an important factor in determining the wetland plants' antioxidant capacities. Our results demonstrated that although phytochemicals in the wetland medicinal plants may contribute significantly to their antioxidant activities, these antioxidant activities were not directly related to the polyphenol quantity. Phytochemicals may play key roles in the potent antioxidant activity of wetland medicinal plants. The potential of these easily accessible sources of natural antioxidants should be explored by the pharmaceutical, medical, and health food industries.

Keywords: Antioxidant; Flavonoid; Flavonol; Polyphenol; Wetland medicinal plant.

INTRODUCTION

It is commonly accepted that reactive oxygen species, such as superoxide ($O_2^{\cdot-}$), hydroxyl ($OH^{\cdot}$), and peroxyl ($^{\cdot}OOH$, $ROO^{\cdot}$) radicals, are produced under oxidative stress. Reactive oxygen species play important roles in degenerative or pathological processes, such as aging (Burns et al., 2001), cancer, coronary heart disease, Alzheimer's disease (Diaz et al., 1997), neurodegenerative disorders, atherosclerosis, diabetes, and inflammation (Chen et al., 2006). Several anti-inflammatory, digestive, anti-necrotic, neuroprotective, and hepatoprotective drugs have recently been shown to have antioxidant and/or radical scavenging mechanisms as well (Lin and Huang, 2002). Some natural antioxidants and compounds with radical scavenging activity have been identified over the last few years, including echinacoside in *Echinaceae* root (Hu and Kitts, 2000), anthocyanin (Espin et al., 2000), phenolic compounds (Rice-Evans et al., 1997), and the extracts of water spinach and sweet potato tuberous roots (Huang et al., 2004; Huang et al., 2005).

Medicinal plant parts are commonly rich in phenolic compounds, such as flavonoids, phenolic acids, stilbenes, tannins, coumarins, lignans and lignins. These compounds have multiple biological effects including antioxidant activity (Packer et al., 1999). *In vitro* experiments on antioxidant compounds in higher plants show how they protect against oxidation damage by inhibiting or quenching free radicals and reactive oxygen species (Ali et al., 2008). The role of these compounds as potential antioxidants can be inferred by their similarity to synthetic antioxidants of related structures.

The multifarious natural environment of Taiwan harbors abundant plant resources. Many of these plants, including those with therapeutic potential, face endangerment. Therefore, we investigated wetland medicinal plants and analyzed their antioxidant activities. In the present study, we collected 31 medicinal wetland plant species that are widely consumed in Taiwan, prepared their water and methanolic extracts, and analyzed their antioxidant activities and polyphenol contents.

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MATERIALS AND METHODS

Materials

Butylated hydroxytoluene (BHT), Glutathione (GSH), 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 6-Hydroxy-2, 5, 7, 8-tetramethylchroman-2-carboxylic acid (Trolox), potassium peroxodisulfate ($K_2S_2O_8$), Tris (hydroxylmethyl) aminomethane, potassium ferricyanide ($K_3Fe(CN)_6$), ferric chloride ($FeCl_3$), catechin, 2,2'-azinobis-(3-ethylbenzothiazoline)-6-sulphonic acid (ABTS), and other chemicals were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Folin-Ciocalteu solution and 95% ethanol were purchased from Merck Co. (Santa Ana, CA, USA). Thirty-one wetland medicinal plants were collected from Taichung, Nantou, and Hsinchu counties in Taiwan. They were identified and authenticated by Dr. Chao-Lin Kuo, Associate Professor and Chairman, Department of Chinese Medicine Resources, China Medical University, Taichung, Taiwan. The medicinal wetland plants studied were all described in the Catalogue of Medicinal Plant Resources in Taiwan, published by the Committee on Chinese Medicine and Pharmacy, Taiwan Department of Health (Lin, 2003).

Plant materials methanol extracts preparation

Dried whole herbs (100 g for each sample) were macerated with 1L 95% ethanol for 24 hours at room temperature, then filtered and extracted three times. The ethanol extract (3 L) was then evaporated to 10 mL and dried in a vacuum at 40°C. The dried extract was weighed, dissolved in 95% ethanol, and stored at -20°C for further use.

Plant materials water extracts preparation

Dried whole herbs (100 g for each sample) were boiled with 1L distilled water for 1 hour. Filtration and extract collection were performed three times. The resulting decoction was evaporated to 10 mL and dried in a vacuum at 50°C. The dried extract was weighed, dissolved in distilled water, and stored at -20°C for further use. For each extract, the yield was calculated as a percentage of the dry weight of the whole herbs used (100 g) and the quantity of dry mass obtained after extraction (*w/w*).

TEAC antioxidant activity determination

A TEAC assay was conducted based on the method of Ramos et al. (1999). The ABTS aqueous solution (7 mM) was oxidized with potassium peroxodisulfate (2.45 mM) for 16 hours in the dark at room temperature. The $ABTS^{\cdot+}$ solution was diluted with 95% ethanol to an absorbance of 0.75 ± 0.05 at 734 nm (Beckman UV-Vis spectrophotometer, Model DU640B). An aliquot (20 μL) of each sample (125 μg/mL) was mixed with 180 μL $ABTS^{\cdot+}$ solution and the absorbance was read at 734 nm after 1 min. Trolox was used as a reference standard. A standard curve was constructed for Trolox at 0, 15.625, 31.25, 62.5, 125, 250, 500 μM concentrations. TEAC value was expressed as millimolar concentration of Trolox solution, with the antioxidant equivalent to a 1000 ppm solution of the sample under investigation.

DPPH radical scavenging antioxidant activity determination

The effects of crude extracts and positive controls (GSH and BHT) on DPPH radicals were estimated based on the method of Yamaguchi et al. (1998). Aliquots (20 μL) of crude extracts at various concentrations were each mixed with 100 mM Tris-HCl buffer (80 μL, pH 7.4) and then with 100 μL of DPPH in ethanol to a final concentration of 250 μM. The mixture was shaken vigorously and left to stand at room temperature for 20 min in the dark. The absorbance of the reaction solution was measured spectrophotometrically at 517 nm. The percentage of DPPH decolorization of the samples was calculated according to the equation: % decolorization = $[1- (ABS_{sample} /ABS_{control})] \times 100$. IC_{50} value was the effective concentration at which DPPH radicals were scavenged by 50% and was obtained by interpolation from linear regression analysis. A lower IC_{50} value indicated a greater antioxidant activity.

Reducing power measurement

The reducing power of the crude extracts and positive controls (GSH and BHT) were determined according to the method of Yen and Chen (1995). The samples (0, 31.25, 62.5, 125, 250, 500, and 1000 μg/mL) were each mixed with an equal volume of 0.2 M phosphate buffer, pH 6.6, and 1% potassium ferricyanide. The mixture was incubated at 50°C for 20 min before an equal volume of 1% TCA was added, and then centrifuged at 5,000 *g* for 10 min. The upper layer of the solution was mixed with distilled water and 0.1% $FeCl_3$ with a ratio of 1: 1: 2, and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated an increase in reducing power.

Total polyphenol content determination

Total polyphenol contents of the crude extracts were determined according to the method of Ragazzi and Veronese (1973). 20 μL of each extract (125 μg/mL) was added to 200 μL distilled water and 40 μL of Folin-Ciocalteu reagent. The mixture was allowed to stand at room temperature for 5 min and then 40 μL of 20% sodium carbonate was added to the mixture. The resulting blue complex was then measured at 680 nm. Catechin was used as a standard for the calibration curve. The polyphenol content was calibrated using the linear equation based on the calibration curve. The total polyphenol content was expressed as mg catechin equivalent/g dry weight. The dry weight indicated was the sample dry weight.

Total flavonoid content determination

Total flavonoid contents of the crude extracts were determined according to the method of Lamaison and Carnet (1990). Aliquots of 1.5 mL extracts were each added to an equal volume of 2% $AlCl_3 \cdot 6H_2O$ (2 g in 100 mL

methanol) solution. The mixture was vigorously shaken, and the absorbance was read after 10 min of incubation at 430 nm. Rutin was used as the standard for the calibration curve. The total flavonoid content was calibrated using the linear equation based on the calibration curve. The total flavonoid content was expressed as mg rutin equivalent/g dry weight. The dry weight indicated was the sample dry weight.

Total flavonol content determination

The total flavonol contents of the crude extracts were determined according to the method of Arnous et al. (2001). Aliquots of 200 μL extracts were each added to 1 mL of 0.1% *p*-dimethylaminocinnamaldehyde (DMACA) in methanol/HCl (3:1, v/v). The mixture was vigorously shaken, and the absorbance was read after 10 min of incubation at 640 nm. Catechin was used as the standard for the calibration curve. The total flavonol content was calibrated using the linear equation based on the calibration curve. The total flavonol content was expressed as mg catechin equivalent/g dry weight. The dry weight indicated was the sample dry weight.

Statistical analysis

Experimental results were presented as the mean ± standard deviation (SD) of three parallel measurements. Statistical analyses were performed by one-way ANOVA, followed by Dunnett's *t* test. The difference was considered to be statistically significant when the *p* value was less than 0.05.

RESULTS

Extraction yields

The water and methanol extract yields of the wetland medicinal plants are presented in Table 1. The water extract yields ranged from 4.24% to 70.18%, and the of methanol extract yields ranged from 0.89% to 34.03%. Among the water extracts, *Pistia stratiotes* produced the highest yield (70.18%), followed by *Lindernia antipoda*. (63.33%), *Polygonum plebeium* (45.99%), *Alisma orientalis* (43.33%), and *Torulinium odoratum* (39.83 %). The highest yield among methanol extracts was obtained from *Lindernia antipoda* (34.03%), followed by *Cyperus alternifolius* subsp. *flabelliformis* (26.76%), *Avicennia marina* (24.37%), *Pistia stratiotes* (21.58%) and *Cyperus difformis* (17.84%).

Water extractions of the wetland medicinal plants generally yielded more components than methanol extractions. It is worth mentioning that water extraction may allow more hydrogen bonding with phenolic compounds than does methanol.

TEAC assay antioxidant activity estimation

The Trolox-equivalent antioxidant capacity (TEAC) assay is often used to evaluate the total antioxidant power of single compounds and complex mixtures of various plants (Chang et al., 2007a, b). In this assay, ABTS radical monocation was generated directly in stable form from potassium peroxodisulfate. The radicals were generated before the addition of antioxidants to prevent the interference of compounds, which affected radical formation. This modification made the assay less susceptible to interruptions and prevented the overestimation of antioxidant power

Table 1. The yield of water and methanol extracts of the wetland medicinal plants.

Scientific name	Yield (%, w/w)[a]	
	Water extract	Ethanol extract
Acorus gramineus Soland.	12.96	8.37
Avicennia marina (Forsk.) Vierh. -leaf	5.47	5.83
Avicennia marina (Forsk.) Vierh. -root	17.84	24.37
Alisma orientalis (Sam.) Juzep.	43.33	14.84
Alternanthera sessilis (L.) R. Br.	15.91	12.14
Cyperus alternifolius L. subsp. *flabelliformis* (Rottb.) Kukenthal	11.24	26.76
Commelina communis L.	11.76	14.21
Cyperus difformis L.	23.22	17.84
Cyperus imbricatus Retz.	36.33	9.79
Cyperus iria L.	11.07	7.62
Eichhornia crassipes (Mart.) Solms	14.35	13.05
Echinochloa crus-galli (L.) Beauv.	6.77	6.39
Egeria densa Planch.	20.77	3.42
Euryale ferox Salisb.	14.44	1.43
Eriocaulon sexangulare L.	12.45	2.53
Fimbristylis littoralis Gaud	10.52	3.92
Hedyotis corymbosa (L.) Lam.	21.19	10.66
Hygrophila pogonocalyx Hayata	12.84	6.41
Juncus effusus L. var. *decipiens* Buchen.	13.04	11.34
Kyllinga brevifolia Rottb.	12.18	4.45
Lindernia antipoda (L.) Alston	63.33	34.03
Marsilea minuta L.	37.03	11.27
Pilea microphylla (L.) Liebm.	4.96	10.76
Phyla nodiflora (L.) Greene	7.86	9.18
Polygonum plebeium R. Br.	45.99	17.31
Pistia stratiotes L.	70.18	21.58
Rotala rotundifolia (Wallich *ex* Roxb.) Koehne	4.24	12.31
Spirodela punctata G. F. W. Meyer	13.96	11.42
Salix warburgii O. Seem.	14.63	15.44
Typha orientalis Presl	19.7	0.89
Torulinium odoratum (L.) S. Hooper	39.83	12.76

[a] On dried weight basis.

(Sanchez-Moreno, 2002). The tested samples were only added to the reaction medium once stable absorbance was obtained. Their antioxidant activities were then measured in terms of decolorization. This method is recommended for plant extracts because the maximum wavelength absorption of ABTS at 734 nm eliminates color interference (Awika et al., 2003). The results were expressed as μM Trolox/mg dry weight of plant material.

In the TEAC assay, the antioxidant capacities of wetland medicinal plants ranged from 7.52 μM to 1753.41 μM Trolox/mg for the water extracts, and 5.69 μM to 2074.35 μM Trolox/mg for the methanol extracts (Table 2). The differences in antioxidant capacities were very large, up to 233 and 364 fold, respectively. Among the water extracts, *Rotala rotundifolia* possessed the highest antioxidant capacity (1753.41 ± 76.99 μM Trolox/mg), followed by *Juncus effusus* var. *decipiens* (971.14 ± 49.68 μM Trolox/mg), *Cyperus iria* (762.04 ± 33.80 μM Trolox/mg), *Salix warburgii* (657.57 ± 18.37 μM Trolox/mg) and *Kyllinga brevifolia* (462.67 ± 9.49 μM Trolox/mg). The plant with the highest antioxidant capacity among the methanol extracts was *Juncus effusus* var. *decipiens* (2074.35 ± 116.19 μM Trolox/mg), followed by *Salix warburgii* (931.45 ± 84.14 μM Trolox/mg), *Cyperus iria* (769.41 ± 53.57 μM Trolox/mg), *Typha orientalis* (651.22 ± 14.95 μM Trolox/mg) and *Kyllinga brevifolia* (342.52 ± 10.91 μM Trolox/mg).

Scavenging activity against 1,1-diphenyl-2-picrylhydrazyl radicals

The relatively stable organic radical DPPH is widely used in modeling systems to investigate the scavenging

Table 2. The TEAC of the water and methanol extracts of the wetland medicinal plants.

Scientific name and positive controls	TEAC[a] (μM Trolox/mg ± SD)	
	Water extracted	Methanol extracted
GSH	1827.68 ± 76.84	Not detected
BHT	Not detected	11869.41 ± 34.63
Acorus gramineus Soland.	159.52 ± 2.57	225.65 ± 9.45
Avicennia marina (Forsk.) Vierh. -leaf	376.17 ± 10.42	54.15 ± 2.11
Avicennia marina (Forsk.) Vierh. -root	381.85 ± 13.07	177.00 ± 2.13
Alisma orientalis (Sam.) Juzep.	12.33 ± 3.44	19.08 ± 7.96
Alternanthera sessilis (L.) R. Br.	156.38 ± 9.48	148.46 ± 6.64
Cyperus alternifolius L. subsp. *flabelliformis* (Rottb.) Kukenthal	47.23 ± 3.66	81.77 ± 4.10
Commelina communis L.	113.02 ± 1.96	96.13 ± 7.69
Cyperus difformis L.	132.00 ± 4.83	15.33 ± 16.20
Cyperus imbricatus Retz.	112.35 ± 4.74	27.35 ± 4.81
Cyperus iria L.	762.04 ± 33.80	769.41 ± 53.57
Eichhornia crassipes (Mart.) Solms	102.13 ± 2.66	143.33 ± 6.39
Echinochloa crus-galli (L.) Beauv.	448.98 ± 6.41	39.56 ± 20.28
Egeria densa Planch.	37.10 ± 2.17	5.69 ± 7.58
Euryale ferox Salisb.	14.58 ± 1.11	350.73 ± 4.13
Eriocaulon sexangulare L.	77.58 ± 3.57	222.65 ± 0.86
Fimbristylis littoralis Gaud	311.73 ± 3.71	87.17 ± 5.02
Hedyotis corymbosa (L.) Lam.	283.58 ± 4.45	56.73 ± 7.18
Hygrophila pogonocalyx Hayata	69.40 ± 0.94	43.38 ± 5.03
Juncus effusus L. var. *decipiens* Buchen.	971.14 ± 49.68	2074.35 ± 116.19
Kyllinga brevifolia Rottb.	462.67 ± 9.49	342.52 ± 10.91
Lindernia antipoda (L.) Alston	320.35 ± 2.80	311.23 ± 16.05
Marsilea minuta L.	94.27 ± 4.82	196.25 ± 13.50
Pilea microphylla (L.) Liebm.	165.44 ± 0.38	248.17 ± 34.50
Phyla nodiflora (L.) Greene	97.04 ± 1.53	86.21 ± 12.58
Polygonum plebeium R. Br.	364.04 ± 1.07	175.44 ± 9.47
Pistia stratiotes L.	7.52 ± 3.64	34.79 ± 2.63
Rotala rotundifolia (Wallich *ex* Roxb.) Koehne	1753.41 ± 76.99	159.90 ± 15.52
Spirodela punctata G. F. W. Meyer	360.25 ± 7.70	104.17 ± 5.36
Salix warburgii O. Seem.	657.57 ± 18.37	931.45 ± 84.14
Typha orientalis Presl	344.13 ± 5.48	651.22 ± 14.95
Torulinium odoratum (L.) S. Hooper	32.71 ± 0.71	203.67 ± 52.57

[a]Values represented mean ± S.D. of three parallel measurements (P<0.05).

activities of several natural compounds, such as phenolics and anthocyanins, as well as crude mixtures, such as methanol or water extracts from plants. The DPPH radical is scavenged by antioxidants through the donation of electrons forming the reduced DPPH. The color changes from purple to yellow after reduction, and the accompanying decrease in absorbance can be quantified at wavelength 517 nm. Table 3 shows the IC_{50} values for radical-scavenging activities of GSH, BHT and different extract fractions of the wetland medicinal plants using the DPPH colorimetric method.

In the DPPH assay conducted on the water extracts, *Rotala rotundifolia* had the lowest IC_{50} value among the medicinal plants (94.89 ± 0.31 μg/mL), followed by *Salix warburgii* (112.69 ± 0.28 μg/mL), *Lindernia antipoda* (189.14 ± 4.55 μg/mL), *Cyperus iria* (194.45 ± 0.32 μg/mL), *Avicennia marina* -leaf (271.71 ± 1.28 μg/mL), and *Polygonum plebeium* (301.52 ± 4.62 μg/mL). The positive control glutathione (GSH) had an IC_{50} value of 71.77 ± 2.09 μg/mL.

For the methanol extracts, *Salix warburgii* had the lowest IC_{50} value (59.58 ± 0.33 μg/mL), followed by *Juncus effusus* var. *decipiens* (108.95 ± 4.47 μg/mL), *Lindernia antipoda* (144.61 ± 2.53 μg/mL), *Cyperus iria* (167.18 ± 0.64 μg/mL), *Typha orientalis* (208.01 ± 1.46 μg/mL), and *Cyperus imbricatus* (242.55 ± 3.11 μg/mL). The positive control BHT also had a low IC_{50} value (139.56 ± 2.96 μg/mL). The above IC_{50} values showed that *Salix warburgii* and *Juncus effusus* var. *decipiens* demonstrated even higher radical scavenging activities than the positive control in the DPPH assay.

Table 3. The DPPH radical scavenging activity of the water and methanol extracts of the wetland medicinal plants.

Scientific name and positive controls	DPPH radical scavenging activity[a] (IC_{50}, μg/mL)	
	Water extract	Methanol extract
GSH	71.77 ± 2.09	Not detected
BHT	Not detected	139.56 ± 2.96
Acorus gramineus Soland.	896.90 ± 7.60	1045.51 ± 0.69
Avicennia marina (Forsk.) Vierh. -leaf	271.71 ± 1.28	>2,000
Avicennia marina (Forsk.) Vierh. -root	404.19 ± 1.18	713.99 ± 0.24
Alisma orientalis (Sam.) Juzep.	>2,000	>2,000
Alternanthera sessilis (L.) R. Br.	844.69 ± 6.42	946.79 ± 8.39
Cyperus alternifolius L. subsp. *flabelliformis* (Rottb.) Kukenthal	>2,000	>2,000
Commelina communis L.	>2,000	>2,000
Cyperus difformis L.	1125.67 ± 1.22	489.04 ± 3.82
Cyperus imbricatus Retz.	1882.64 ± 3.92	242.55 ± 3.11
Cyperus iria L.	194.45 ± 0.32	167.18 ± 0.64
Eichhornia crassipes (Mart.) Solms	>2,000	>2,000
Echinochloa crus-galli (L.) Beauv.	548.23 ± 4.62	>2,000
Egeria densa Planch.	>2,000	>2,000
Euryale ferox Salisb.	>2,000	307.35 ± 1.61
Eriocaulon sexangulare L.	>2,000	>2,000
Fimbristylis littoralis Gaud	810.61 ± 6.58	>2,000
Hedyotis corymbosa (L.) Lam.	668.89 ± 8.62	>2,000
Hygrophila pogonocalyx Hayata	1520.06 ± 5.25	>2,000
Juncus effusus L. var. *decipiens* Buchen.	456.88 ± 3.88	108.95 ± 4.47
Kyllinga brevifolia Rottb.	379.52 ± 2.52	523.55 ± 0.091
Lindernia antipoda (L.) Alston	189.14 ± 4.55	144.61 ± 2.53
Marsilea minuta L.	1400.48 ± 3.2	613.76 ± 1.67
Pilea microphylla (L.) Liebm.	>2,000	423.14 ± 5.61
Phyla nodiflora (L.) Greene	>2,000	789.26 ± 5.84
Polygonum plebeium R. Br.	301.52 ± 4.62	>2,000
Pistia stratiotes L.	>2,000	>2,000
Rotala rotundifolia (Wallich *ex* Roxb.) Koehne	94.89 ± 0.31	721.89 ± 3.91
Spirodela punctata G. F. W. Meyer	432.20 ± 4.63	1094.73 ± 12.61
Salix warburgii O. Seem.	112.69 ± 0.28	59.58 ± 0.33
Typha orientalis Presl	533.59 ± 4.92	208.01 ± 1.46
Torulinium odoratum (L.) S. Hooper	>2,000	>2,000

[a]Values represented mean ± S.D. of three parallel measurements (P<0.05).

Reducing power measurement

We investigated the reducing capacity of wetland medicinal plants by measuring Fe^{3+}-Fe^{2+} conversion. The reducing capacity of a compound may serve as an important indicator of its potential antioxidant activity (Meir et al., 1995). The antioxidant activities of putative antioxidants have been attributed to various mechanisms, such as the prevention of chain initiation, transition metal ion catalyst binding, peroxides decomposition , prevention of continued proton abstraction, and radical scavenging (Diplock, 1997). The reducing power of different extract fractions from the wetland medicinal plants are shown in Table 4. Both reduced GSH and BHT were used as the positive controls.

For the reducing capacity of the water extracts, *Salix warburgii* had the highest value among the medicinal plants examined (1.64 ± 0.01, Δ700), followed by *Rotala rotundifolia* (1.61 ± 0.05, Δ700), *Lindernia antipoda* (1.60 ± 0.07, Δ700), *Cyperus iria* (1.56 ± 0.01, Δ700), *Polygonum plebeium* (1.17 ± 0.03, Δ700), and *Avicennia marina*.-leaf

Table 4. The reducing power of the water and methanol extracts of the wetland medicinal plants.

Scientific name and positive controls	Reducing power Δ700[a] (Mean ± SD)	
	Water extract	Methanol extract
GSH	1.80 ± 0.01	Not detected
BHT	Not detested	0.27 ± 0.02
Acorus gramineus Soland.	0.36 ± 0.01	0.04 ± 0.01
Avicennia marina (Forsk.) Vierh. -leaf	1.11 ± 0.01	0.06 ± 0.01
Avicennia marina (Forsk.) Vierh. -root	1.010 ± 0.02	0.36 ± 0.02
Alisma orientalis (Sam.) Juzep.	0.05 ± 0.01	0.15 ± 0.02
Alternanthera sessilis (L.) R. Br.	0.46 ± 0.01	0.19 ± 0.02
Cyperus alternifolius L. subsp. *flabelliformis* (Rottb.) Kukenthal	0.13 ± 0.01	0.17 ± 0.01
Commelina communis L.	0.23 ± 0.01	0.40 ± 0.01
Cyperus difformis L.	0.39 ± 0.01	0.51 ± 0.02
Cyperus imbricatus Retz.	0.27 ± 0.02	0.41 ± 0.03
Cyperus iria L.	1.56 ± 0.01	0.58 ± 0.03
Eichhornia crassipes (Mart.) Solms	0.17 ± 0.01	0.26 ± 0.01
Echinochloa crus-galli (L.) Beauv.	0.63 ± 0.02	0.03 ± 0.01
Egeria densa Planch.	0.02 ± 0.01	0.04 ± 0.01
Euryale ferox Salisb.	0.07 ± 0.01	0.76 ± 0.12
Eriocaulon sexangulare L.	0.09 ± 0.01	0.32 ± 0.01
Fimbristylis littoralis Gaud	0.40 ± 0.01	0.01 ± 0.01
Hedyotis corymbosa (L.) Lam.	0.552 ± 0.02	0.06 ± 0.00
Hygrophila pogonocalyx Hayata	0.310 ± 0.01	0.09 ± 0.02
Juncus effusus L. var. *decipiens* Buchen.	0.56 ± 0.01	0.26 ± 0.05
Kyllinga brevifolia Rottb.	0.74 ± 0.01	0.29 ± 0.08
Lindernia antipoda (L.) Alston	1.60 ± 0.07	1.66 ± 0.01
Marsilea minuta L.	0.27 ± 0.01	0.69 ± 0.02
Pilea microphylla (L.) Liebm.	0.08 ± 0.03	0.29 ± 0.02
Phyla nodiflora (L.) Greene	0.33 ± 0.02	0.36 ± 0.03
Polygonum plebeium R. Br.	1.17 ± 0.03	0.36 ± 0.02
Pistia stratiotes L.	0.02 ± 0.01	0.04 ± 0.01
Rotala rotundifolia (Wallich *ex* Roxb.) Koehne	1.61 ± 0.05	0.25 ± 0.01
Spirodela punctata G. F. W. Meyer	0.71 ± 0.05	0.10 ± 0.04
Salix warburgii O. Seem.	1.64 ± 0.01	1.68 ± 0.01
Typha orientalis Presl	0.58 ± 0.01	0.46 ± 0.02
Torulinium odoratum (L.) S. Hooper	0.09 ± 0.01	0.27 ± 0.05

[a]Values represented mean ± S.D. of three parallel measurements (P<0.05).

(1.11 ± 0.01, Δ700). The positive control glutathione (GSH) had a high reducing capacity activity of 1.80 ± 0.01, Δ700.

For the methanol extracts, *Salix warburgii* had the highest reducing capacity value (1.68 ± 0.01, Δ700), followed by *Lindernia antipoda* (1.66 ± 0.01, Δ700), *Euryale ferox* (0.76 ± 0.12, Δ700), *Marsilea minuta* (0.69 ± 0.02, Δ700), *Cyperus iria* (0.58 ± 0.03, Δ700), and *Cyperus difformis* (0.51 ± 0.02, Δ700). The positive control BHT also had quite a high reducing capacity (0.27 ± 0.02, Δ700). The results showed that the reducing capacities for radical-scavenging of all the tested wetland medicinal plants were even higher than those of the positive controls.

Total polyphenol, flavonoid, and flavonol contents of the wetland medicinal plants

The total polyphenol, flavonoid, and flavonol contents of the water and methanol extracts of wetland medicinal plants are shown in Tables 5 and 6, respectively. The total polyphenol content is expressed as μg of catechin equivalent per milligram of dry weight. For the water extracts, the total polyphenol content of the wetland medicinal plants ranged from 17.91 μg to 565.92 μg CE/mg; as for the methanol extract, the total polyphenol content ranged from 7.69 μg CE/mg to 551.50 μg CE/mg, and the difference of antioxidant capacities was also very large, up to 31 and 71 fold. *Rotala rotundifolia* had a total polyphenol content of 565.92 ± 4.45 μg CE/mg, followed by *Lindernia antipoda* (389.25 ± 19.12μg CE/mg), *Cyperus iria* (385.67 ± 5.62 μg CE/mg), *Cyperus iria* (302.89 ± 21.19 μg CE/mg), *Typha orientalis* (230.50 ± 1.41 μg CE/mg), and *Cyperus difformis* (162.04 ± 10.16 μg CE/mg) in their water extracts (Table 5). *Salix warburgii* had a total polyphenol content of 551.50 ± 17.57 μg CE/mg, followed by *Typha orientalis* (494.17 ± 10.13 μg CE/mg), *Juncus effusus*. var. *decipiens* (489.75 ± 53.28 μg CE/mg), *Lindernia antipoda* (356.70 ± 17.32 μg CE/mg), *Cyperus iria* (345.25 ± 9.81 μg CE/mg), and *Kyllinga brevifolia* (251.25 ± 1.90 μg CE/mg) in their methanol extracts (Table 6).

The total flavonoid content was expressed as μg of rutin equivalent per milligram of dry weight. The total flavonoid contents in the wetland medicinal plant water extracts ranged from 3.11 to 53.92 μg RE/mg, and the total flavonoid contents in their methanol extracts ranged from 1.19 to 72.17 μg RE/mg, furthermore the difference of antioxidant capacities was also very large, up to 17 and 60 fold respectively. *Typha orientalis* had the highest total flavonoid content (53.92 ± 5.44 μg RE/mg), followed by *Rotala rotundifolia* (46.24 ± 0.39 μg RE/mg), and *Cyperus iria* (29.73 ± 0.51 μg RE/mg) in their water extracts. *Eriocaulon sexangulare* had the highest total flavonoid content (74.55 ± 1.50 μg RE/mg), followed by *Polygonum plebeium* (72.17 ± 3.33 μg CE/mg), *Typha orientalis* (71.89 ± 0.42 μg RE/mg), and *Salix warburgii* (70.34 ± 2.43 μg RE/mg) in their methanol extracts.

The total flavonol content was expressed as μg of catechin equivalent per milligram of dry weight. The total flavonol content of the wetland medicinal plants water extracts ranged from 0.05 to 14.05 μg CE/mg, and that of the methanol extracts ranged from 0.46 to 14.36 μg CE/mg. The difference in antioxidant capacities was also very large, from 31 up to up to 281 fold. *Cyperus iria* had the highest flavonol content (14.05 ± 0.88 μg CE/mg), followed by *Polygonum plebeium* (13.47 ± 1.22 μg CE/mg), and *Salix warburgii* (5.54 ± 0.18 μg CE/mg) for the water extracts. *Cyperus imbricatus* had the highest flavonol content (14.36 ± 1.28 μg CE/mg), followed by *Cyperus iria* (13.41 ± 0.87 μg CE/mg), and *Typha orientalis* (7.04 ± 0.10 μg CE/mg) for the methanol extracts.

Relationship between total antioxidant activity and total polyphenol content

The correlation coefficients (R^2) of total antioxidant activity (TEAC) and total polyphenols of the water and methanol extracts are shown in Figure 1A and 1B. The R^2 values of TEAC and total polyphenol content of the water (Figure 1A) and methanol (Figure 1B) extracts were 0.14 and 0.25, respectively. From these statistics, we determined a low correlation between the TEAC and total polyphenol contents. Linear regression analysis showed a low correlation between antioxidant activity and total phenolic contents. Different wetland medicinal plant species may influence the antioxidant activity as well. High phenolic content is only one of the antioxidant capacity indicators.

DISCUSSION

The best antioxidant activities among the 31 wetland medicinal plants screened, based on TEAC assay, DPPH radical scavenging, reducing power, total polyphenol content, total flavonoid content and flavonol content results were: *Rotala rotundifolia*, *Juncus effusus* var. *decipiens*, *Cyperus iria*, *Salix warburgii*, *Lindernia antipoda*, *Kyllinga brevifolia*, and *Typha orientalis*. The therapeutic properties of *Rotala rotundifolia* have not been reported in any scientific papers before. This plant possesses antiradiation, anti-inflammatory, and antibacterial properties. The present study provided valuable preliminary data through a demonstration of its efficient antioxidant capacity. Isolation and characterization of its individual active components and *in vivo* relevance await further comprehensive studies.

Juncus effusus var. *decipiens* possesses anti-depressant, anti-inflammatory, and antibacterial effects. To our knowledge, there were no prior reports on the antioxidant activity of this plant. This study rendered valuable preliminary data through demonstration of its high antioxidant capacity. To study the phenolic constituents from the dry stem of *Juncus effusus*, six phenolic constituents were purified and identified as 7-carboxy-2-hydroxy-1-methyl-5-vinyl-9, 10-dihydrophenanthrene, 2,3-isopylidene-1-O-ferulic acid glyceride, (2S)-2, 3-isopylidene-1-0-p-coumaroyl glyceride, dehydroeffusal, p-hydroxybenzaldehyde and luteolin-5,3'-dimethyl ether (Li et al., 2007). Some of these might be antioxidants.

Table 5. Total polyphenol, flavonoid, and flavonol content of the water extracts of the wetland medicinal plants[a].

Scientific name	Water extracted		
	Polyphenol[a, b] (μg CE/mg)	Flavonoid[a, c] (μg RE/mg)	Flavonol[a, b] (μg CE/mg)
Acorus gramineus Soland.	91.50 ± 1.25	13.81 ± 4.93	2.06 ± 0.14
Avicennia marina (Forsk.) Vierh. -leaf	109.25 ± 1.67	27.55 ± 0.24	0.34 ± 0.02
Avicennia marina (Forsk.) Vierh. -root	102.1 ± 7.55	7.78 ± 0.07	0.42 ± 0.01
Alisma orientalis (Sam.) Juzep.	37.04 ± 1.81	3.71 ± 0.05	1.06 ± 0.01
Alternanthera sessilis (L.) R. Br.	137.67 ± 3.81	26.44 ± 0.53	1.56 ± 9.11
Cyperus alternifolius L. subsp. *flabelliformis* (Rottb.) Kukenthal	78.45 ± 0.93	4.63 ± 0.05	1.23 ± 0.02
Commelina communis L.	113.79 ± 5.76	15.72 ± 0.20	1.04 ± 0.01
Cyperus difformis L.	162.04 ± 10.16	18.96 ± 0.53	1.15 ± 0.01
Cyperus imbricatus Retz.	140.87 ± 7.01	13.70 ± 0.37	1.31 ± 0.01
Cyperus iria L.	385.67 ± 5.62	29.73 ± 0.51	14.05 ± 0.88
Eichhornia crassipes (Mart.) Solms	90.12 ± 7.26	9.97 ± 0.23	1.14 ± 0.01
Echinochloa crus-galli (L.) Beauv.	108.33 ± 9.26	14.88 ± 1.33	2.93 ± 1.98
Egeria densa Planch.	24.66 ± 0.10	5.85 ± 0.09	1.05 ± 0.01
Euryale ferox Salisb.	28.16 ± 0.49	3.28 ± 0.21	1.93 ± 0.02
Eriocaulon sexangulare L.	88.62 ± 0.91	9.57 ± 0.25	1.25 ± 0.02
Fimbristylis littoralis Gaud	87.50 ± 20.02	14.16 ± 0.36	3.13 ± 0.08
Hedyotis corymbosa (L.) Lam.	157.50 ± 7.53	17.04 ± 0.67	0.71 ± 0.01
Hygrophila pogonocalyx Hayata	81.25 ± 9.11	7.86 ± 0.08	1.22 ± 0.01
Juncus effusus L. var. *decipiens* Buchen.	37.33 ± 10.37	8.79 ± 2.88	0.38 ± 0.01
Kyllinga brevifolia Rottb.	215.92 ± 1.23	9.54 ± 0.44	1.69 ± 0.01
Lindernia antipoda (L.) Alston	389.25 ± 19.12	22.68 ± 0.57	2.23 ± 0.02
Marsilea minuta L.	102.41 ± 6.93	12.42 ± 0.61	3.97 ± 0.15
Pilea microphylla (L.) Liebm.	81.67 ± 3.59	10.00 ± 0.40	0.05 ± 0.01
Phyla nodiflora (L.) Greene	137.04 ± 4.13	16.76 ± 0.10	1.61 ± 0.01
Polygonum plebeium R. Br.	64.10 ± 10.48	17.22 ± 0.25	13.47 ± 1.22
Pistia stratiotes L.	17.91 ± 0.10	3.33 ± 0.04	0.85 ± 0.01
Rotala rotundifolia (Wallich *ex* Roxb.) Koehne	565.92 ± 4.45	46.24 ± 0.39	4.50 ± 0.27
Spirodela punctata G. F. W. Meyer	67.67 ± 11.58	21.37 ± 1.27	0.81 ± 0.02
Salix warburgii O. Seem.	302.89 ± 21.19	34.20 ± 1.36	5.54 ± 0.18
Typha orientalis Presl	230.50 ± 1.41	53.92 ± 5.44	2.89 ± 0.01
Torulinium odoratum (L.) S. Hooper	43.91 ± 0.55	3.11 ± 0.04	2.39 ± 0.04

[a]Values represented mean ± S.D. of three parallel measurements.
[b]Data expressed in μg catechin equivalent / mg dry weight (μg CE/mg).
[c]Data expressed in μg rutin equivalent / mg dry weight (μg RE/mg).

Cyperus iria has not been reported in any scientific papers before. This plant possesses rheumatic, antidiuretic, and anti-inflammatory effects. The present study showed first-hand data on the antixodiant capacity of *Cyperus iria*. Isolation and characterization of its individual active components and *in vivo* relevance of such activity await further comprehensive studies.

Salix warburgii possesses anticoagulant, anti-inflammatory, and antibacterial properties. To our knowledge, there have been no prior reports on the antioxidant activity of this plant. This paper studied the antioxidant effects of *Salix warburgii* for the first time; a bioassay-guided *in vitro* screen has revealed that a 70% methanol extract of the leaves of *Salix matsudana* showed considerable inhibitory activity against cyclooxygenases (COX-1 and COX-2) (Li et al., 2008). A subsequent phytochemical study led to the isolation of some compounds: matsudone A, luteolin, isoquercitrin, 7-methoxyflavone, luteolin 7-O-

Table 6. Total polyphenol, flavonoid, and flavonol content of the methanol extracts of the wetland medicinal plants [a].

Scientific name	Methanol extracted		
	Polyphenol[a, b] (μg CE/mg)	Flavonoid[a, c] (μg RE/mg)	Flavonol[a, b] (μg CE/mg)
Acorus gramineus Soland.	160.58 ± 61.15	19.98 ± 31.55	1.38 ± 0.01
Avicennia marina (Forsk.) Vierh. -leaf	7.69 ± 24.50	18.34 ± 24.74	1.49 ± 0.16
Avicennia marina (Forsk.) Vierh. -root	59.76 ± 3.27	42.08 ± 1.22	0.46 ± 0.01
Alisma orientalis (Sam.) Juzep.	37.45 ± 0.28	6.25 ± 1.93	0.94 ± 0.01
Alternanthera sessilis (L.) R. Br.	152.83 ± 11.30	24.07 ± 18.78	3.79 ± 0.38
Cyperus alternifolius L. subsp. *flabelliformis* (Rottb.) Kukenthal	105.58 ± 13.19	20.51 ± 3.02	3.84 ± 0.05
Commelina communis L.	129.16 ± 4.99	21.21 ± 0.24	1.36 ± 0.01
Cyperus difformis L.	39.01 ± 6.05	15.31 ± 0.68	2.29 ± 0.02
Cyperus imbricatus Retz.	112.07 ± 10.79	26.11 ± 9.91	14.36 ± 1.28
Cyperus iria L.	345.25 ± 9.81	46.88 ± 0.47	13.41 ± 0.87
Eichhornia crassipes (Mart.) Solms	184 ± 19.12	25.60 ± 6.30	3.08 ± 0.06
Echinochloa crus-galli (L.) Beauv.	80.08 ± 8.80	24.21 ± 10.61	2.00 ± 0.38
Egeria densa Planch.	81.79 ± 18.52	12.5.11 ± 5.41	2.95 ± 0.01
Euryale ferox Salisb.	213.58 ± 7.29	16.70 ± 2.31	3.43 ± 0.01
Eriocaulon sexangulare L.	133.02 ± 5.12	74.55 ± 1.50	6.15 ± 0.86
Fimbristylis littoralis Gaud	107.08 ± 4.12	19.16 ± 5.42	2.00 ± 0.37
Hedyotis corymbosa (L.) Lam.	103.67 ± 1.46	26.02 ± 6.49	2.57 ± 0.16
Hygrophila pogonocalyx Hayata	18.05 ± 8.56	1.19 ± 0.53	2.54 ± 1.34
Juncus effusus L. var. *decipiens* Buchen.	489.75 ± 53.28	30.16 ± 5.07	1.60 ± 0.05
Kyllinga brevifolia Rottb.	251.25 ± 1.90	41.86 ± 2.19	3.85 ± 0.26
Lindernia antipoda (L.) Alston	356.70 ± 17.32	35.21 ± 2.42	2.12 ± 0.01
Marsilea minuta L.	47.81 ± 9.58	12.05 ± 3.84	1.45 ± 0.08
Pilea microphylla (L.) Liebm.	244.08 ± 14.43	14.78 ± 3.38	5.98 ± 0.78
Phyla nodiflora (L.) Greene	29.53 ± 4.71	11.10 ± 1.86	2.68 ± 0.10
Polygonum plebeium R. Br.	44.32 ± 11.38	72.17 ± 3.33	2.82 ± 0.39
Pistia stratiotes L.	51.79 ± 1.12	20.22 ± 2.58	4.97 ± 0.12
Rotala rotundifolia (Wallich *ex* Roxb.) Koehne	122.92 ± 3.67	28.14 ± 5.48	2.43 ± 0.33
Spirodela punctata G. F. W. Meyer	204.00 ± 4.39	20.84 ± 1.53	3.77 ± 0.29
Salix warburgii O. Seem.	551.50 ± 17.57	70.34 ± 2.43	6.62 ± 0.31
Typha orientalis Presl	494.17 ± 10.13	71.89 ± 0.42	7.04 ± 0.10
Torulinium odoratum (L.) S. Hooper	54.06 ± 11.80	12.27 ± 2.27	3.33 ± 1.56

[a]Values represented mean ± S.D. of three parallel measurements.
[b]Data expressed in μg catechin equivalent / mg dry weight (μg CE/mg).
[c]Data expressed in μg rutin equivalent / mg dry weight (μg RE/mg).

glucoside, and 4',7-dihydroxyflavone. These isolated compounds were found to possess activities in inhibiting against COX-1 or COX-2.

Lindernia antipoda possesses analgesic and anti-inflammatory effects. There have been no prior reports on the antioxidant activity of this plant. This study provided valuable data by demonstrating the high antioxidant capacity of *Lindernia antipoda* for the first time. However, isolation and characterization of its active components and their *in vivo* relevance await further comprehensive studies.

Kyllinga brevifolia possesses analgesic and anti-inflammatory effects. Oral administration of doses up to 3.0 g/kg did not provoke any toxic symptoms. The toxicity of this plant was observed to be dose dependent and its intraperitoneal LD_{50} was found to be 575 mg/kg. It is used in traditional medicine to alleviate stress or as a sedative agent (Helliön-Ibarrola et al., 1999).

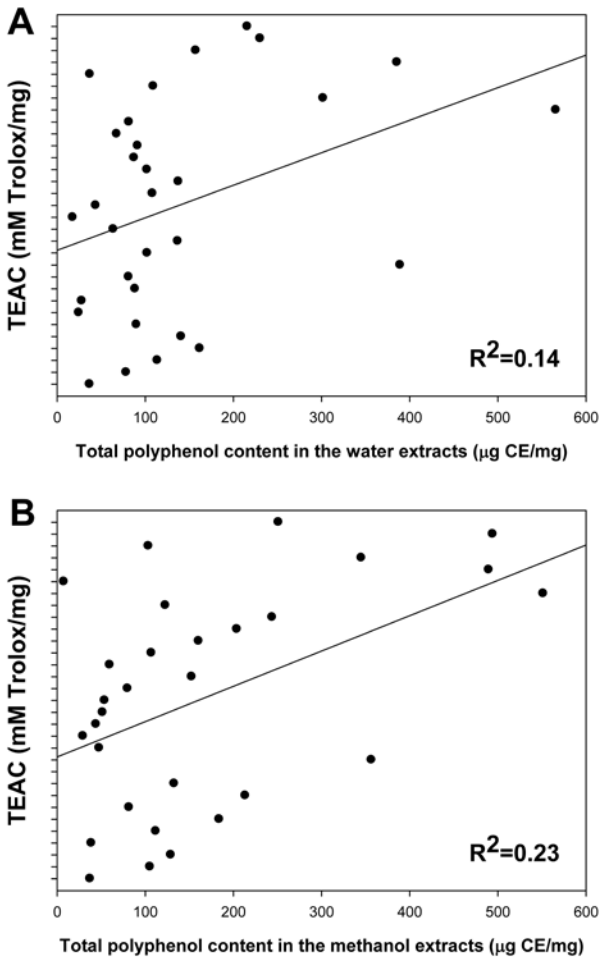

Figure 1. Correlation coefficients (R^2) of TEAC and total polyphenol contents in the water (A) and methanol (B) extracts of the wetland medicinal plants.

Typha orientalis is a commonly used Chinese herbal drug which has been shown to possess blood circulation stimulating, hypertension relieving and nerve soothing effects. There are no prior reports on the antioxidant activity of this plant.

Phenolic compounds, such as flavonoids, phenolic acid and tannins, possess anti-inflammatory, anti-carcinogenic, anti-atherosclerotic, and other properties that may be related to their antioxidant activities (Chung et al., 1998; Wong et al., 2006). Flavonoids and flavonols are two polyphenolic compounds that play an important role in stabilizing lipid oxidation and are associated with antioxidant activity (Yen et al., 1993). Phenolic compounds may contribute directly to antioxidative action (Duh et al., 1999). Polyphenolic compounds may have an inhibitory effect on mutagenesis and carcinogenesis in humans when as much as 1.0 g is ingested daily from a diet rich in fruits and vegetables (Tanaka et al., 1998). The antioxidative activities observed can be attributed to both the different mechanisms exerted by different phenolic compounds and to the synergistic effects of different compounds. The antioxidant assays used in this study measured the oxidative products at the early and final stages of oxidation. Antioxidants have different functional properties, for example quercetin, rutin, and catechin can scavenge reactive oxygen species (Liu et al., 2008); *p*-coumaric acids, on the other hand, inhibit the generation of free radicals and chain-breaking activity (Laranjinha et al., 1995) and metal chelation (Van-Acker et al., 1998). These compounds, as well as flavonoids and other organic acids, are highly effective electron donors. However, the components responsible for the antioxidative activities of the wetland medicinal plants are still unclear. Further work must be performed to isolate and identify these components.

In conclusion, the results from these *in vitro* experiments, including ABTS radical monocation scavenging (Table 2), DPPH radical scavenging (Table 3), reducing power method (Table 4), total polyphenol content, total flavonoid content and total flavonol content (Table 5 and 6), demonstrated that phytochemicals in wetland medicinal plants might have significant effects on antioxidant activities. However, the quantity of polyphenols and flavonoids found in the wetland medicinal plant extracts were not directly related to their antioxidant activities. The additive roles of phytochemicals might contribute significantly to the potent antioxidant activity. Hence, some wetland medicinal plants could be used as an easy accessible source of natural antioxidants in pharmaceutical and medical industries. For this reason, further work should be performed to isolate and identify the antioxidative components of wetland medicinal plants.

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LITERATURE CITED

Ali, B.H., G. Blunden, M.O. Tanira, and A. Nemmar. 2008. Some phytochemical, pharmacological and toxicological properties of ginger (*Zingiber officinale* Roscoe): a review of recent research. Food Chem. Toxicol. **46:** 409-420.

Arnous, A., D.P. Makris, and P. Kefalas. 2001. Effect of principal polyphenolic components in relation to antioxidant characteristics of aged red wines. J. Agric. Food Chem. **49:** 5736-5742.

Awika, R., P. Wu, J.M. Cisneros-Zevallos, L.W. Awika, X.L. Rooney, R.L. Wu, and L. Cisneros-Zevallos. 2003. Screening methods to measure antioxidant activity of sorghum (*Sorghum bicolor*) and sorghum products, J. Agric. Food Chem. **51:** 6657-6662.

Burns, J., P.T. Gardner, D. Matthews, G.G. Duthie, M.E. Lean, and A. Crozier. 2001. Extraction of phenolics and changes in antioxidant activity of red wines during vinification. J. Agric. Food Chem. **49:** 5797-5808.

Chang, H.C., G.J. Huang, D.C. Agrawal, C.L. Kuo, C.R. Wu, and H.S. Tsay. 2007b. Antioxidant activities and polyphenol contents of six folk medicinal ferns used as "Gusuibu". Bot. Stud. **48:** 397-406.

Chang, H.Y., Y.L. Ho, M.J. Sheu, Y.H. Lin, M.C. Tseng, S.H. Wu, G.J. Huang, and Y.S. Chang. 2007a. Antioxidant and free radical scavenging activities of *Phellinus merrillii* extracts. Bot. Stud. **48:** 407-417.

Chen, F.A., A.B. Wu, P. Shieh, D.H. Kuo, and C.Y. Hsieh. 2006. Evaluation of the antioxidant activity of *Ruellia tuberosa*. Food Chem. **94:** 14-18.

Chung, K.T., T.Y. Wong, Y.W. Huang, and Y. Lin. 1998. Tannins and human health: a review, Critical Reviews in Food Science and Nutrition. **38:** 421-464.

Diaz, M.N., B. Frei, J.A. Vita, and J.F. Keaney. 1997. Antioxidants and atherosclerotic heart disease. N. Engl. J. Med. **337:** 408-416.

Diplock, A.T. 1997. Will the 'good fairies' please prove to us that vitamin E lessens human degenerative disease? Free Radic. Res. **27:** 511-532.

Duh, P.D., Y.Y. Tu, and G.C. Yen. 1999. Antioxidant activity of water extract of Harng Jyur (*Chyrsanthemum morifolium* Ramat). LWT. **32:** 269-277.

Espin, J.C., C. Soler-Rivas, H.J. Wichers, and C. Viguera-Garcia. 2000. Anthocyanin-based natural colorants: a new source of antiradical activity for foodstuff. J. Agric. Food Chem. **48:** 1588-1592.

Helliön-Ibarrola, M.C., D.A. Ibarrola, Y. Montalbetti, D. Villalba, O. Heinichen, and E.A. Ferro. 1999. Acute toxicity and general pharmacological effect on central nervous system of the crude rhizome extract of Kyllinga brevifolia Rottb. J. Ethnopharmacol. **66:** 271-276.

Hu, C. and D.D. Kitts. 2000. Studies on the antioxidant activity of *Echinaceae* root extract. J. Agric. Food Chem. **48:** 1466-1472.

Huang, D.J., C.D. Lin, H.J. Chen, and Y.H. Lin. 2004. Antioxidant and antiproliferative activities of sweet potato (*Ipomoea batatas* [L.] Lam 'Tainong 57') constituents. Bot. Bull. Acad. Sin. **45:** 179-186.

Huang, D.J., H.J. Chen, C.D. Lin, and Y.H. Lin. 2005. Antioxidant and antiproliferative activities of water spinach (*Ipomoea aquatica* Forsk) constituents. Bot. Bull. Acad. Sin. **46:** 99-106.

Lamaison, J.L.C. and A. Carnet. 1990. Teneurs en principaux flavonoids des fleurs de *Crataegeus monogyna* Jacq et de *Crataegeus laevigata* (Poiret D. C) en fonction de la vegetation. Pharm. Acta. Helv. **65:** 315-320.

Laranjinha, J., O. Vieira, V. Madeira, and L. Almeida. 1995. Two related phenolic antioxidants with opposite effects on vitamin E content in low density lipoproteins oxidized by ferrylmyoglobin: consumption versus regeneration. Arch. Biochem. Biophys. **323:** 373-381.

Li, H.X., T.Z. Deng, Y. Chen, H.J. Feng, and G.Z. Yang. 2007. Isolation and identification of phenolic constituents from *Juncus effuses*. Yao Xue Xue Bao. **42:** 174-178.

Li, X., Z. Liu, X.F. Zhang, L.J. Wang, Y.N. Zheng, C.C. Yuan, and G.Z. Sun. 2008. Isolation and characterization of phenolic compounds from the leaves of *Salix matsudana*. Molecules **13:** 1530-1537.

Lin, C.C. and P.C. Huang. 2002. Antioxidant and hepatoprotective effects of *Acathopanax senticosus*. Phytother. Res. **14:** 489-494.

Lin, I.H. 2003. The Catalogue of Medicinal Plant Resources in Taiwan, Committee on Chinese Medicine and Pharmacy, Department of Health, Taipei, Taiwan.

Liu, C.L., Y.S. Chen, J.H. Yang, and B.H. Chiang. 2008. Antioxidant activity of tartary (*Fagopyrum tataricum* (L.) Gaertn.) and common (*Fagopyrum esculentum* Moench) buckwheat sprouts. J. Agric. Food Chem. **56:** 173-178.

Meir, S., J. Kanner, B. Akiri, and S.P. Hadas. 1995. Determination and involvement of aqueous reducing compounds in oxidative defense systems of various senescing leaves. J. Agric. Food Chem. **43:** 1813-1817.

Packer, L., G. Rimbach, and F. Virgili. 1999. Antioxidant activity and biologic properties of a procyanidin-rich extract from pine (*Pinus maritima*) bark, pycnogenol. Free Radic. Biol. Med. **27:** 704-724.

Ragazzi, E. and G. Veronese. 1973. Quantitative analysis of phenolics compounds after thin-layer chromatographic separation. J. Chromatogr. **77:** 369-375.

Ramos, F., Y. Takaishi, K. Kawazoe, C. Osorio, C. Duque, R. Acuña, Y. Fujimoto, R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, and C. Rice-Evans. 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med. **26:** 1231-1237.

Rice-Evans, C.A., N.J. Miller, and G. Paganga. 1997. Antioxidant properties of phenolic compounds. Trends Plant Sci. **2:** 152-159.

Sanchez-Moreno, C. 2002. Methods used to evaluate the free radical scavenging activity in foods and biological systems. Food Sci. Technol. Int. **8:** 121-137.

Tanaka, M., C.W. Kuei, Y. Nagashima, and T. Taguchi. 1998. Application of antioxidative maillrad reaction products from histidine and glucose to sardine products. Nippon Suisan Gakkai Shil. **54:** 1409-1414.

Van-Acker, S.A.B.E., G.P. Van-Balen, D.J. Vanden-Berg, A. Bast, and S.A.B.E. Vander-Vijgh. 1998. Influence of iron chelation on the antioxidant activity of flavonoids. Biochem. Pharmacol. **56:** 935-943.

Wong, C.C., H.B. Li, K.W. Cheng, and F. Chen. 2006. A systematic survey of antioxidant activity of 30 Chinese medicinal plants using the ferric reducing antioxidant power assay. Food Chem. **97:** 705-711.

Yamaguchi, T., H. Takamura, T. Matoba, and J. Terao. 1998.

HPLC method for evaluation of the free radical-scavenging activity of foods by using 1,1,-diphenyl-2-picrylhydrazyl. Biosci. Biotechnol. Biochem. **62:** 1201-1204.

Yen, G.C. and H.Y. Chen. 1995. Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem. **46:** 849-854.

Yen, G.C., P.D. Duh, and C.L. Tsai. 1993. Relationship between antioxidant activity and maturity of peanut hulls. J. Agric. Food Chem. **41:** 67-70.

臺灣濕地藥用植物之抗氧化活性和總多酚含量

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本文章研究目的是評估台灣濕地藥用植物之甲醇和水萃取物之抗氧化活性。評估的項目，包括ABTS 清除，清除 DPPH 自由基，還原力，總多酚含量，總類黃酮類含量、總黃酮醇類含量。結果顯示，31 種濕地藥用植物中以水豬母乳、燈心草、碎米莎草、水柳、泥花草、短葉水蜈蚣和香蒲共七種，其抗氧化物和多酚類均具不錯之效果和含量。且由抗氧化活性和總多酚含量之線性相關係數結果得知，水萃取物相關係數為 0.14 和甲醇萃取物相關係數為 0.23。結果顯示，植物中化學物質含量可能有助於顯著的抗氧化活性，但這種關係並不一定成正比。濕地藥用植物未來在醫藥和保健食品行業中將可作為一個容易取得的天然抗氧化劑的來源。

關鍵詞：濕地藥用植物；抗氧化劑；多酚類；黃酮類；黃酮醇類。