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Antioxidant Activities of Methanol Extracts from Selected Taiwanese Herbaceous Plants

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Abstract The purpose of this study was to evaluate the total phenolic and flavonoid contents in acid methanol extracts of 19 plant species grown and used in Taiwan. The antioxidant activity of leafy plant extracts was determined by measuring the trolox equivalent antioxidant capacity (TEAC), oxygen radical absorption capacity (ORAC), and 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity. Polyphenol, flavonol, and flavonoid levels were high in *Sonchus oleraceus* (SO), *Ohwia caudate* Thunb. Ohashi (OC), *Foeniculum vulgare* Mill. (FV), and *Artemisia princeps* Pamp. var. orientalis (Pamp.) Hars. (ArP, fresh) as compared to the rest of tested plants. In addition, quercetin, myricetin, and morin were abundant in *Dendranthe mamori folium* (Ramat.) Tzvelev. (DM, dry), FV, SO, *Alternanthera philoxeroides* (Moq.) Griseb. (AlP), ArP (fresh) and *Lonicera japonica* Thunb. (LJ). Higher levels of the TEAC, ORAC, and DPPH radical scavenger were generated from extracts of OC, SO, DM (dry), and ArP (fresh). Significant and positive correlations among antioxidant activity and polyphenols and anthocyanidins were observed. ORAC values were also correlated with quercitin and morin. Therefore, these phytochemicals were some of the main components responsible for the antioxidant efficacy of tested plants.

Keywords: *herbaceous plants, TEAC, ORAC, polyphenol, flavonol*

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1. Introduction

Plants such as herbs have long been used in traditional medicine in various cultures throughout the world. Over 100 plant species are consumed worldwide as vegetables, but only about 20 of them are grown globally and account for most of the vegetables produced and consumed [1].Various types of plants have been used not only for dietary supplements but also as traditional folk treatments for many health problems [2].The use of folk medicine is widespread and herbaceous plants comprise an important source of bioactive compounds possessing potent biological properties. They have played a significant role in traditional medicine since ancient times and still represent an important source of natural antioxidants that might lead to the development of novel drugs [3].

Antioxidant activity is a fundamental property important for human life. Many biological functions, including anti-mutagenicity, anti-carcinogenicity, and anti-aging, may originate from this property [4]. The increased consumption of herbaceous plants has been widely promoted because of the health benefits of many non-nutrient phytochemicals associated with health maintenance and prevention of chronic diseases and cancers. As our understanding of the role of free radicals in human diseases has deepened, antioxidants have attracted broader interest because of their role in inhibiting free radical reactions and their help in protecting the human body against damage by reactive oxygen species [5]. However, herbaceous plants differ in the types and levels of antioxidants they contain. The synergies and antagonisms of antioxidants in crude mixtures add complexity in attempts to explain their antioxidant capacity.

Some phenolics are ubiquitous compounds found in all plants as secondary metabolites [6]. Numerous groups of phytochemicals in herbaceous plants are recognized for their antioxidant activity [7].Crude extracts of fruits, herbs, vegetables, cereals, and other plant materials are rich in phenols and are increasingly of interest to the food industry because they retard the oxidative degradation of lipids and thereby improve the quality and nutritional value of food [8]. For example, *Centella asiatica* (CA) has been subjected to intensive pharmacological investigations in recent years because it shows a wide spectrum of action, including antioxidant, antibacterial, and antitumor activity [9,10,11]. *Lycium chinense* (LC) protects the liver damage, mainly by decreasing oxidation [12], although it is usually used in treating diabetes. Phenolic compounds found in *Bidens pilosa* (BP) have antioxidant effects, therefore, their ingestion may help to prevent *in vivo* oxidative

damage, like that which occurs in lipid peroxidation in association with cancer and premature ageing [13]. Previously, we found that that *Plantago asiatica* (PA), BP, and *Mesona procumbens* (MP) are rich in polyphenols, and methanolic extracts of PA, BP, CA, *Mentha crispa* (MC), and *Mentha arvensis* (MA) have higher reducing power and DPPH radical scavenging activity as compared to other test plant species [14]. Moreover, acidic methanolic extracts of MA and *Houttuynia cordata* (HC) significantly suppressed oxidative damage to lymphocyte DNA [15]. Over the past decade, we have evaluated more than 50 edible plant samples representing highly consumed and under-utilized plant species using Trolox equivalent antioxidant capacity (TEAC) and 1,1-Diphenyl-2-picryl-hydrazyl (DPPH) radical scavenging activity methods to investigate the antioxidant activity of their water- and methanol-soluble antioxidants in plant extracts [14-19].

Landrace herbaceous plants are widely used throughout Taiwan in ready-made preparations and herbal drugs, and have provided a foundation for traditional pharmaceuticals and drug leads [14,15]. Taiwan has a great diversity of landrace plant species and hence is a potential source for bioactive compounds [14]. The 19 landrace herbaceous plants selected for this study contain various phytochemicals and are commonly used as herbal medicines for antioxidant, anti-inflammation, anti-bacterial and anticancer purposes in Taiwan [9,10,11]. Although a number of these species have been used for food flavoring and traditional medicine, insufficient research has been done on their antioxidant properties. This study focuses on 19 different edible landrace plant species known to play important roles in the diets of Taiwan's inhabitants.

Although the tested plants in this study are associated with some biological functions, further scientific evidence is still required in many cases to verify the effects. The objective of this study was to isolate, identify, and evaluate the antioxidant components and antioxidant activity of different extracts from19 selected herbaceous plant species. Our study explores the relationships between the composition and content of flavonols and polyphenols having antioxidant efficiency. This is the first report of the antioxidant contents of 18 of the 19 plant species (excepting PA) analyzed in this study. These plants may have excellent potential as functional ingredients representing potential sources of natural antioxidants.

2. Materials and Methods

2.1. Chemicals and Reagents

Methanol, ethanol, acetone, hydrochloric acid, formic acid, sodium chloride (NaCl), di-sodium hydrogen phosphate, potassium dihydrogen phosphate, Trolox, and butylated hydroxyltoluene (BHT) were purchased from Merck (Darmstadt, Germany). Gallic acid, peroxidase, H_2O_2, sodium carbonate (Na_2CO_3), 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonicacid) (ABTS), aluminium chloride ($AlCl_3$), sodium acetate, Folin-Ciocalteau reagent, 2,2-diphenyl-1-picrylhydrazyl (DPPH), methylated β-cyclodextrin (RMCD), 2,2-azobis (2-methylpropionamidine) dihydrochloride (AAPH), fluorescein (FL), myricetin, morin, quercetin, kaempferol, cyanidin, malvidin, delphinidin, and pelargonidin were procured from Sigma Chemical (St Louis, MO, USA).

2.2. Plant Materials and Preparation of Plant Extracts

The 19 Taiwan herbaceous landrace plants were used as shown in Table 1.

Table 1. 19 Taiwan herbaceous landrace plants

Scientific name	Abreviation
Alternanthera philoxeroides (Moq.) Griseb.	AlP
Amaranthus viridis	AV
Artemisia princeps Pamp. var. orientalis (Pamp.) Hars.	ArP, fresh and powder
Cardamine flexuosa With.	CF
Ceratopteris thalictroides Brongn.	CT
Cordia dichotoma Forst. f.	CD, leaf
Crassocephalum crepidioides (benth.) S. Moore.	CC
Dendranthe mamori folium (Ramat.) Tzvelev.	DM, fresh and dry
Foeniculum vulgare Mill.	FV
Gynura formosana Kitamura	GF, wild
Hedychium coronarium Koening	HC
Lablab purpureus Sweet	LP
Lonicera japonica Thunb.	LJ
Ohwia caudate Thunb. Ohashi	OC
Plantago asiatica	PA
Portulaca oleracea	PO
Sonchus oleraceus	SO
Talinum triangulare Willd.	TT
Zanthoxylum ailanthoides Sieb. and Zucc.	ZA

All plant materials originated from and were grown in Taiwan, and were purchased from different local supermarkets in Taipei City, Taiwan. Leaves of samples were divided into eight individual batches, weighed, lyophilized, ground to powder, and stored at – 80°C until use. Extracts were prepared according to Harnly et al. [20]. Briefly, 5 g of powder was refluxed at 75°C for 5 h in 50 mL of acidified methanol (1.2 N HCl) with 0.4 g/L BHT and filtered through Whatman grade no. 1 qualitative filter paper. Extracts were then concentrated in a rotary vacuum evaporator (R205, Buchi, Flawil, Switzerland), re-suspended in acidic methanol to 6 mL, and stored at–20°C until testing for antioxidants with high-performance liquid chromatography (HPLC).

2.3. Determinations of Polyphenols, Total Flavonols, Total Flavonoids, and Anthocyanidins

Polyphenol content was determined according to the method of Taga et al. [21]. Briefly, standard gallic acid and an aliquot of the acidic methanolic extract were diluted with acidified methanol solution containing 1% HCl. Two mL of 2% Na_2CO_3 were mixed into each sample of 100 μL and allowed to equilibrate for 2 min before adding 50% Folin-Ciocalteu reagent. Absorbance at 750 nm was measured at room temperature using the Varioskan Flash Multimode Reader (Thermo Scientific, Rockford, IL, USA). The standard curve for gallic acid was used to calculate polyphenol levels. Total phenolics were expressed as the mg gallic acid equivalent (GAE)/g of dry weight. Total flavonols in plant extracts were

estimated based on Kumaran and Joel Karunakaran [22]. Briefly, 80% of ethanol containing 1% HCl of solvent was used to extract the lyophilized vegetable samples in a shaker for 2 h at room temperature, then centrifuged at 1430 *g* for 15 min at 4°C, and repeated extraction for three times. Two mL of 2% $AlCl_3$ ethanol and 3.0 mL (50 g/L) sodium acetate were added to 2.0 mL acidic ethanolic extracts. Absorption at 440 nm was read after 2.5 h at 25°C Sample extracts were evaluated at a final concentration of 0.1 mg/mL. Total flavonols were calculated as a quercetin equivalent (mg/g) and expressed as the mg quercetin equivalent (QUE) mg/g dry weight. Total flavonoids were determined using the method of Ordonez et al. [23]. Briefly, 0.5 mL of 2% $AlCl_3$-ethanol solution was added to 0.5 mL of acidic ethanolic extract and absorbance was measured at 420 nm after standing for 1 h at room temperature. Extract samples were evaluated at a final concentration of 0.1 mg/mL. Total flavonoids were calculated as a quercetin equivalent (mg/g) and expressed as QUE. Anthocyanidins were determined by the method of Mancinelli et al. [24]. Briefly, an acidified methanol solution (contained 1% HCl) was used to extract lyophilized plant samples in a shaker for2 h at room temperature followed by centrifuging at 4°C under 1430 *g* for 15 min and repeated twice. Supernatants were then measured at absorbances of 657 nm and 530 nm. Briefly, a solution of 99% methanol and 1% HCl was used to extract lyophilized plant samples in a shaker for 1 h at room temperature followed by centrifuging at 4°C under 1430 *g* for 15 min and repeated three times. Supernatants were then measured at absorbances of 657 nm and 530 nm. Anthocyanidins (unit/g) = (A_{530} - 0.33 A_{657}) x mL of extraction/g DW.

2.4. Flavonols and Anthocyanidins Analysis

One mL of acid hydrolysates methanolic extract was passed through a 0.45 μm filter prior to injecting 20 mL of it into an HPLC. Samples were analyzed with a Hitachi D-2000 containing a Photodiode Array Detector (L-2455) (Hitachi High-Tech, Tokyo, Japan) and an ODS column (250 × 4.6 mm, 5 μm; YMC, ODS-A, Kyoto, Japan). The mobile phase consisted of acetonitrile-water (30%:70%; v/v) containing 1% phosphate acid. The eluent was 100% in 30 min at a flow rate of 1.00 mL/min. The spectrum was recorded at 365 nm for flavonols determination [25]. Two running solvents were delivered at a rate of 1 mL/min: solvent A was 90% water and 10% formic acid, and solvent B was 22.5 % acetonitrile, 22.5% methanol, 40% water, and 10% formic acid. The gradient program used was as follows: 0 min, 20% of solvent B; 0.1~30 min, 28% of solvent B; 30.1~40 min, 70% of solvent B; 40.1~45 min, 100% of solvent B [19]. The spectrum was recorded at 520 nm for anthocyanidins determination. Flavonols (myricetin, morin, quercetin, and kaempferol) and antocyanidins (delphinidin, cyanidin, pelargonidin, and malvidin) were used as standards

2.5. TEAC, ORAC, and DPPH Radical Assays

The total antioxidant capacity of hydrophilic and lipophilic antioxidants was determined using the horseradish peroxidase catalyzed oxidation of 2,2-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) [26].The reaction mixture contained 0.5 mL of 1000 μM ABTS (in ddH_2O) and 3.5 mL of 100 μM H_2O_2 (in 0.1 M PBS). The reaction was started by adding 0.5 mL of 44 U/mL peroxidase (in 0.1 M PBS). After 1 h, 0.05 mL of the sample extract was added to the mixture. Absorbance was measured at 730 nm after 10 min. Trolox was used as a standard, and the total antioxidant capacity of the sample extract was measured. The TEAC value was expressed as μmol Trolox per gram dry weight.

For the lipophilic antioxidant assay, the dried hexane extract was dissolved in 250 μL of acetone and then diluted with 750 μL 7% RMCD solution (50% acetone to 50% water, v/v) [27]. Any further dilution was with the 7% RMCD solution. The 7% RMCD solution was also used as a blank and to dissolve Trolox standards for the lipophilic assay. For sample extract lipophilic analysis, 20 μL of this solution was added to each well in a 96-well microplate. Two hundred μL of fluorescein solution was added to the microplate reader followed by 75 μL of 63.4 mM AAPH (17.2 mg/mL and 9.4 μmol/well), and readings were initiated immediately. Any further dilution of the hydrophilic fraction (acetone/water/acetic acid extract) during the ORAC assay was made with the phosphate buffer. A 20-μL portion of the diluted sample extract was added to each well in a 96-well microplate. Fluorescein solution and AAPH were added in the same manner as in the lipophilic assay, except that 75 μL of 31.7 mM AAPH was added to the hydrophilic assay mixture [28].

The scavenging activity of the DPPH radical in sample extracts was determined according to Shimada et al. [29]. Briefly, an aliquot of 1 mL of a methanolic extract with series dilution was added to 1 mL of 0.8 mM DPPH freshly prepared in methanol, mixed well, and left to stand for 30 min before measuring absorbance at 517 nm. The scavenging effect percentage was calculated as [1 − (OD_{517}nm)/(control OD_{517}nm)] × 100. The $IC_{50}$of the scavenging effect percentage was then calculated.

2.6. Statistical Analysis

Data is expressed as the mean±standard deviation, and statistical significance was analyzed using a one-way analysis of variance (ANOVA) followed by Tukey's Range Test at the 0.05 significance level [30]. Pearson's correlation coefficients between antioxidant substances and capacities were also determined [30]. The means of three replicates are reported.

3. Results and Discussion

3.1. Antioxidant Content

Table 2 documents the content of various antioxidant substances in the leaves of tested plants. SO and OC contained significantly higher polyphenol levels (29.80 ± 1.65 and 26.24 ± 0.96 mg gallic acid/g DW, respectively) than the other species. Flavonols were abundant in FV, GF, ArP (powder), and AV at levels >40 mg quercetin equivalent/g DW. The leaves of DM (dry), FV, HC, and ArP (fresh) were significantly rich in flavonoids, ranging from 11.54 ± 2.21 to 15.49 ± 2.44 mg quercetin equivalent/g DW. In general, DM (dry), FV, ArP (fresh), and OC contained abundant polyphenols, flavonols, and

flavonoids. Anthocyanidins were the principal antioxidants in OC (83.39± 3.63 unit/g DW) and LP (43.94± 1.22 unit/g DW). However, the leaves of FV, CC, PO, TT, CF, AlP, and AV were not rich in anthocyanidins. Table 2 lists the flavonol contents in acidic ethanolic extracts of plant leaves. DM (dry) was rich in quercetin (18009.58 ± 328.91 μg/g DW), while DM (fresh) and SO followed at levels of 10915.12 ± 550.88 and 10315.36 ± 539.84 μg/g DW, respectively). Myricetin was abundant in FV (1283.16 ± 60.13 μg/g DW) compared to other plants. Interestingly, AV and OC only contained quercetin (139.45 ± 9.03 μg/g DW) and myricetin (80.27 ± 18.41 μg/g DW), respectively. However, quercetin and myricetin were not detected in GF, CT, LP, or PA. Samples from ArP (fresh), ArP (powder), and LJ (14,521.52 ± 492.51, 16,517.43 ± 599.75, and 14,764.31 ± 502.53 μg/g DW, respectively) contained significantly higher levels of morin than other samples. CT and PA contained only morin (1218.54 ± 306.18 and 604.99 ± 27.58 μg/g DW, respectively). Kaempferol was present only in ZA (47.96 ± 5.99 μg/g DW, the highest level), GF, and DM (4.29 ± 1.43 μg/g DW, the lowest level), but the other plants did not contain any kaempferol at all. Furthermore, no flavonols were detected in LP. Thus, these Taiwanese landrace plants display a wide variation in flavonol levels. Among the tested 19 species, only LP and OC contained anthocyanidins, namely delphinidin (Del), cyanidin (Cyan), and malvidin (Mal) (data not shown). Both Cyan and Mal were abundant in LP and OC, and OC had the highest levels of Cyan and Mal at 650.44 ± 1.34 μg/g DW and 40.02 ± 2.98 μg/g DW, respectively (data not shown). Interestingly, Del was detected only in OC, and none of the tested plants contained pelargonidin (data not shown).

Table 2. The content of various antioxidant substances in acid methanolic hydrolysates of Taiwanese landrace plants

Sample	Polyphenols (mg gallic acid /g DW)	Flavonols (mg quercetin equivalent/g DW)	Flavonoids (mg quercetin equivalent/g DW)	Anthocyanidins (unit/g DW)
AlP	15.17±0.41 de	30.26±4.87 d	10.49±1.88 c	N.D.
ArP	24.22±0.99 b	35.43±5.20 c	11.54±2.21 bc	3.74±0.40 h
ArP(*)	17.94±0.81 d	44.04±3.49 ab	9.54±2.47 cd	12.75±1.05 d
AV	13.66±0.57 f	40.92±3.35 b	8.13±1.24 cd	N.D.
CC	19.51±0.32 c	38.28±5.35 bc	7.61±1.57 d	N.D.
CD	13.71±0.57 ef	38.53±3.41 b	8.18±1.48 cd	6.01±0.45 f
CF	14.81±0.17 e	33.13±5.84 cd	5.86±2.10 e	N.D.
CT	13.76±0.10 f	23.00±4.34 ef	7.39±1.29 d	4.12±0.14 g
DM	16.48±1.42 d	18.22±1.40 g	7.92±0.80 d	7.90±0.48 ef
DM(*)	19.41±0.32 c	31.33±1.20 cd	11.90±2.37 bc	8.86±0.15 e
FV	18.87±0.56 cd	46.41±4.21 a	15.49±2.44 a	N.D.
GF	14.08±0.22 e	41.91±2.82 b	10.31±2.42 c	2.42±1.34 ij
HC	20.10±1.67 c	23.05±2.00 f	13.23±0.52 b	17.00±0.26 c
LJ	20.51±0.28 c	24.85±2.15 e	7.13±2.56 d	3.58±0.13 h
LP	12.09±0.35 g	8.97±1.21 h	3.33±0.50 f	43.94±1.22 b
OC	26.24±0.96 ab	38.98±1.82 b	8.29±2.50 cd	83.39±3.63 a
PA	14.85±0.38 e	35.78±3.23 c	6.93±0.38 de	2.65±0.71 i
PO	16.50±1.15 d	30.06±5.08 d	5.84±1.56 e	N.D.
SO	29.80±1.65 a	26.01±5.31 e	8.11±0.55 cd	5.50±0.44 fg
TT	17.60±1.18 d	39.03±4.66 b	6.15±1.15 de	N.D.
ZA	19.35±0.63 c	30.29±3.65 d	7.21±1.72 d	1.16±0.32 j

All values are means ± standard deviations (n=3). Means within a column with different letters (a~j) are significantly different, $p < 0.05$.
DW, dry weight. ND, not detected.
*Samples of DM and ArP were purchased in dry and powder form and used in each antioxidant assay.

Table 3. Flavonols content in acidic methanolic hydrolysates from Taiwanese landrace plants

	Flavonols (μg/g DW)			
Sample	Quercetin	Myricetin	Morin	Kaempferol
AlP	1543.00±77.47 g	809.27±32.22 b	820.24±146.16 ij	N.D.
ArP	3742.04±596.00 d	N.D.	14521.52±492.51 a	N.D.
ArP (*)	3090.80±261.31 de	N.D.	16517.43±599.75 a	N.D.
AV	139.45±9.03 jk	N.D.	N.D.	N.D.
CC	512.37±23.37 i	N.D.	2335.35±192.05 g	N.D.
CD	2210.40±299.38 ef	N.D.	1138.05±99.85 hi	N.D.
CF	153.01±39.26 j	N.D.	302.67±74.11 l	N.D.
CT	N.D.	N.D.	1218.54±306.18 hi	N.D.
DM	10915.12±550.08 b	279.53±58.98 de	3030.49±108.38 f	6.91±2.30 b
DM (*)	18009.58±328.91 a	357.94±44.60 d	12194.45±429.32 b	4.29±1.43 b
FV	5843.73±2.54 c	1283.16±60.13 a	7775.65±517.74 de	N.D.
GF	N.D.	N.D.	737.39±39.45 j	5.18±1.73 b
HC	102.37±36.30 k	239.75±19.64 e	2157.46±472.47 gh	N.D.
LJ	1905.61±155.02 f	698.16±28.82 c	14764.31±502.53 a	N.D.
LP	N.D.	N.D.	N.D.	N.D.
OC	N.D.	80.27±18.41 g	N.D.	N.D.
PA	N.D.	N.D.	604.99±27.58 k	N.D.
PO	1229.25±67.31 h	N.D.	149.21±52.63 m	N.D.
SO	10315.36±539.84 b	220.28±37.43 e	11177.69±215.82 c	N.D.
TT	43.17±14.39 l	128.03±24.68 f	5975.47±402.07 e	N.D.
ZA	612.37±86.13 i	342.70±59.03 d	8466.83±276.23 d	47.96±5.99 a

All values are means ± S.D. (n=3). Means within a column with different letters (a~m) are significantly different, $p < 0.05$. DW, dry weight. ND, not detected.
*Samples of DM and ArP were purchased in dry and powder form and used in each flavonol assay.

Plants are rich in flavonoids and other pigments. All of the tested plants contained polyphenols, flavonols, and flavonoids. Most samples were found to contain anthocyanidins, quercetin, myricetin, and morin (Tables 2 and 3). Quercetin and morin were the dominant flavonols in the samples. However, kaempferol was not detected in most of the plants due to it being sensitive to acid methanolic hydrolysis and because it rarely occurs in vegetables and other herbaceous plants. Quercetin and morin are important for preventing peroxidation and oxidative damage to DNA [15]. The high levels of quercetin and morin are believed to account for the high DNA protective potential of HC and MA [15]. Anthocyanidins act as strong antioxidants and show activity comparable to antioxidants α-tocopherol and Trolox at a concentration of 10 μM [31]. Generally, medicinal herbs contain significantly higher levels of phenolics than common vegetables and fruits [32]. Furthermore, quercetin, the most abundant flavonoid in the human diet, is an excellent free radical scavenging antioxidant [33]. Previously, we showed that PA, BP, CA, MC, and MA have free radical scavenging activity [14]; moreover, BP, MA, HC, CA, PA, and LC were found to be rich in flavonols, especially myricetin in BP, morin in MA, quercetin in HC, and kaemperol in CA [15]. Recently, we reported the antioxidant components of purple sweet potato leaf extract (PSPLE), quercetin, and cyanidin may down-regulate intracellular redox-dependent signaling pathways in human aortic endothelial cells (HAECs) upon TNF-α stimulation [34]. In the present study, OC contained high levels of polyphenols, flavonols, and anthocyanidins, and also had higher antioxidant activity in term of TEAC and ORAC values. Moreover, SO, ArP (fresh), and OC were abundant in polyphenols while HC, LP, and OC were abundant in anthocyanidins. DM (fresh), DM (dry), FV, and SO had significant levels of quercetin. Xia et al. [35] reported that SO is rich in polyphenols (388.4 mg GAE/g DW) and flavonoids (148.5 mg rutin/g DW). In addition, Jimoh et al. [36] also showed that SO contains polyphenols (9.72 ± 1.06 mg tannic acid equivalents/g DW) and flavonoids (1.21 ± 0.01 mg quercetin/g DW).

3.2. Antioxidant Activity of Methanol and Ethanol Extracts Measured for TEAC and ORAC Values and DPPH Radical Scavenging Activity

Plant extracts from all species show antioxidant activity, proving their capacity for scavenging ABTS radical cations. The antioxidant activity of acidic methanolic hydrolysate and ethanolic extracts of leaf tissue is expressed in TEAC. Methanolic extracts had higher TEAC values than ethanolic extracts in all tested plant species except SO, ArP (fresh), Arp (powder), LJ, and OC, indicating that methanolic extracts were more effective than ethanolic extracts in scavenging ROS (Table 4). For instance, the methanolic extract of HC had a high TEAC (AV) to 15.48 ± 0.10 μg/mL (OC). OC had a significantly higher efficacy of DPPH radical scavenging activity than other samples. Hence, each tested sample showed significant differences in scavenging DPPH at value (124.11 ± 0.32 μmol Trolox/g DW) while the ethanolic extract exhibited only 16.18 ± 2.89 μmol Trolox/g DW. Moreover, the methanolic extract (24.62 ± 1.24 μmol Trolox/g DW) of AV was19-fold higher than the TEAC value of the ethanolic extract (1.24 ± 0.21 μmol Trolox/g DW). However, and interestingly, DM (dry), ArP (fresh), LJ, and OC plants also had higher TEAC values in both extraction solvents.

Cai et al. [32] demonstrated that TEAC values and total phenolic levels of 112 traditional Chinese medicinal plants in methanolic extracts ranged from 0.467 to 173.23 μmol Trolox equivalent/g DW and from 2.2 to 503 mg of gallic acid equivalent/g DW, respectively. A significant linear relationship between antioxidant activity and total phenolic content shows that phenolic compounds are the dominant antioxidant components of these112 medicinal herbs, and the major types of phenolic compounds from most of them included mainly phenolic acids, flavonoids, tannins, coumarins, lignans, quinones, stilbenes, and curcuminoids [32]. In our study, TEAC values and total phenolics in methanolic extracts ranged from 24.62 to 747.48 μmol Trolox equivalent/g DW (Table 4) and from 12.09 to 29.80 mg of gallic acid equivalent/g DW (Table 1), respectively. Our TEAC values were apparently higher than Cai's data [32]. Particularly, FV, PO, ArP, and OC in our study had much higher TEAC-methanolic values and total phenolics in methanolic extracts compared to Cai's data [32]. Part of the antioxidant activity may be due to those phenolic compounds. In addition, antioxidant activity observed in plants could be a synergistic effect of more than two compounds present in the plant [37]. Different phytochemicals may exhibit effective antioxidant activity alone or synergistically, and are a likely cause of species differences. Synergisms among antioxidants make antioxidant activity dependent not only on their concentrations but perhaps also on their structures, plus interactions among the antioxidants [38].

All extracts exhibited distinct antioxidant capacities, with hydrophilic oxygen radical absorption capacity (ORAC) values from 23599.54 ± 477.01 (DM-dry) to 787.48 ± 70.80 (GF) μmol Trolox/g DW (Table 4), while ORAC-lipophilic values ranged from 6922.42 ± 414.72 μmol Trolox/g DW (HC) to 430.00 ± 36.47 μmol Trolox/g DW (CD). Most of the selected landrace plants had higher ORAC-hydrophilic values than ORAC-lipophilic values, indicating that hydrophilic extracts are more effective than lipophilic extracts in scavenging ROS. Compared to some South Asia herbs [39] and Chinese herbs [40], the tested herbs in our study showed higher ORAC values. The ability of antioxidant's strength is to eliminate oxygen free radicals, and both ORAC-hydrophilic and TEAC assays display similar top rankings for extracts, with DM (dry) and OC being the most effective, followed by ArP (fresh). The lowest antioxidant capacities were obtained with GF, CF, and AV.

Plant methanolic extracts exhibited a wide range of DPPH scavenging values from 2413.91 ± 26.19 μg/mL IC_{50} (Table 3). The IC_{50} of SO's DPPH radical scavenging activity was 56.5 μg/mL in the 70% methanolic extract, and SO also contained the highest amounts of phenolics and flavonoids [41]. TR was abundant in quercetin,

myricetin, and kaempferol, and its DPPH radical scavenging IC_{50} was 87.5 μg/mL [42]. Khan et al. [43] showed that *Morus alba* leaf methanolic extract and butylated hydroxytoluene (BHT) DPPH radical scavenging values were 108.69 and 8.5 μg/mL, respectively, while OC leaf methanolic extract DPPH radical scavenging activity was 15.48 μg/mL in our study (Table 4). The effect of antioxidants on DPPH is thought to be due to their hydrogen donating ability [33], and methanolic extracts of these plants have good free radical scavenging ability and can be used as radical inhibitors or scavengers.

Table 4. TEAC values, ORAC values, and IC_{50} of DPPH scavenging activity in acid methanolic hydrolysates and ethanolic extracts of Taiwanese landrace plants

Sample	TEAC-methanolic (μmol Trolox/g DW)	TEAC-ethanolic (μmol Trolox/g DW)	Hydrophilic ORAC (μmol Trolox/g DW)	Lipophilic ORAC (μmol Trolox/g DW)	DPPH scavenge IC_{50} (μg/mL)
AlP	73.97±3.66 fg	5.36±1.26 j	4240.36±211.79 i	1393.79±64.92 hi	112.40±0.05 m
ArP	124.01±2.97 c	138.78±19.16 cd	19465.84±424.39 b	2944.86±491.77de	74.16±0.65 n
ArP (*)	57.26±4.45 h	126.76±16.39 cd	10066.47±159.62 e	3348.34±252.80d	74.87±0.37 n
AV	24.62±1.24 m	1.24±0.21 l	804.93±104.91 kl	803.56±48.58 kl	2413.91±16.19 a
CC	79.06±4.95 f	56.69±1.83 e	6579.52±524.96 f	3965.78±148.86 c	73.20±0.07 n
CD	29.01±3.31 l	9.45±1.00 i	2788.36±212.70 j	430.00±36.47 m	1145.40±0.76 b
CF	38.05±4.36 j	2.25±0.85 k	997.98±134.57 k	1926.95±98.10 g	1119.76±13.62 c
CT	38.72±3.17 j	5.44±0.66 j	951.28±62.45 k	593.68±59.42 l	379.44±0.62 e
DM	30.25±2.16 kl	13.43±1.37 h	13287.10±388.51 d	2429.44±171.20 ef	48.67±0.16 p
DM (*)	163.89±5.66 b	113.46±12.05 d	23599.54±477.01 a	1926.29±123.54 g	58.89±0.37 o
FV	101.88±6.13 e	22.88±3.37 g	10310.58±293.96 e	4727.75±308.04 b	208.28±0.11 i
GF	33.62±0.76 k	5.34±0.39 j	787.48±70.80 l	4760.15±36.46 b	339.17±0.77 f
HC	124.11±0.32 c	16.18±2.89 gh	14366.77±662.36 d	6922.42±414.72 a	257.81±0.78 h
LJ	118.86±4.76 d	161.79±11.60 c	9774.97±188.45 e	1006.98±80.62 j	71.76±0.55 n
LP	47.08±0.24 i	6.97±0.25 j	5700.02±226.54 fg	1002.21±84.52 j	73.57±0.04 n
OC	747.48±19.03 a	759.73±23.88 a	20938.16±807.29 b	1441.15±238.02h	15.48±0.10 q
PA	70.10±6.30 g	15.57±1.27 h	4437.68±590.61 hi	2466.18±454.15e	117.93±0.05 l
PO	121.08±10.62 cd	33.84±2.92 f	5421.68±532.33 gh	1434.73±146.22 h	137.11±2.35 k
SO	79.00±5.43 f	187.27±4.36 b	16956.93±392.85 c	791.58±45.02 kl	846.75±0.14 d
TT	98.26±4.20 e	29.84±4.06 f	3064.94±103.85 j	893.48±45.19 k	265.76±0.53 g
ZA	69.38±1.27 g	33.57±2.08 f	6484.15±401.50 f	1205.78±101.06 i	155.68±0.02 j

All values are means ± S.D. (n=3). Means within a column with different letters (a~q) are significantly different, $p < 0.05$. DW, dry weight. ND, not detected.
*Samples of DM and ArP were purchased in dry and powder form and used in each antioxidant activity assay.

Table 5. Correlation analysis among antioxidant substances and activity versus hydrophilic- and lipophilic-ORAC and TEAC values treated with acid methanolic extracts

Sample	Hydrophilic ORAC (μmol Trolox/g DW)	Lipophilic ORAC (μmol Trolox/g DW)	TEAC-methanolic (μmol Trolox/g DW)
Polyphenols	r = 0.63	NS	r = 0.49
thocyanidins	r = 0.29	NS	r = 0.75
Flavonoids	NS	NS	NS
Flavonols	NS	NS	NS
Quercetin	r = 0.61	NS	NS
Myricetin	NS	NS	NS
Morin	r = 0.54	NS	NS
Kaempferol	NS	NS	NS
DPPH scavenge IC_{50}	r = -0.43	r = -0.30	r = -0.29
TEAC-methanolic	r = 0.60	NS	--
TEAC-ethanolic	r = 0.62	NS	--

Data are presented as correlation coefficients. $P < 0.05$ for each correlation.
NS, non-significance correlation. --, not analyzed.

Correction analysis reveals a significant positive correction between TEAC values and polyphenol levels (r= 0.49) as well as between TEAC values and anthocyanidins (r = 0.75) (Table 5). Hydrophilic ORAC values were positively and significantly correlated with the levels of polyphenols (r = 0.63), quercitin (r = 0.61), and morin (r = 0.54); however, no significant correlations were observed for the rest of the samples. Interestingly, lipophilic-ORAC values were not correlated with any antioxidant substance (Table 5). Thus, different antioxidant substances displayed various levels of antioxidant activity. DPPH radical scavenging IC_{50} values were significantly and negatively correlated with other antioxidant activity assays (Table 4), while both TEAC-methanolic and TEAC-ethanolic values were significantly and positive correlated with hydrophilic-ORAC values (Table 5).

The amounts of polyphenols and anthocyanidins were significantly and positively correlated with observed TEAC values, indicating that phenolic compounds might be major contributors to the antioxidant activity of plant extracts. However, no correlation was found among quercetin, morin, and TEAC, which may be due to the structure required to reinforce the free radical scavenging activity that varies with the type of free radical [44]. González-Paramás et al. [45] demonstrated that flavonol levels were significantly and highly correlated (r = 0.8) with TEAC values. Total phenolic and flavonoid contents

were positively and highly correlated (r= 0.825 and 0.987, respectively) with ORAC values [46]. Cao et al. [47] suggested that the number of -OH groups in the A ring and B ring of flavonoids was strongly correlated with their ORAC values. Taken together, polyphenol-rich plants exhibited distinct cell-free antioxidant activity (TEAC, ORAC, and DPPH radical scavenging) as validated by their polyphenol, flavonoid and flavonol levels, with distinct antioxidant activity strongly accounting for the antioxidant activity of the extracts. Antioxidant activity tests, scavenging of DPPH radicals, and TEAC and ORAC assays were selected because different ROSs have different reaction mechanisms, at least two methods are recommended for evaluating antioxidant activity [37,44]. ORAC is the more biologically relevant assay [37,44], reflecting the major mechanisms of antioxidant action for evaluating relevance to cell protection. TEAC values for common fruits and vegetables regularly consumed in Western countries correlate with values measured by other widely used methods, including ORAC and total phenolics [48]. Ou et al. [28] found large variations within vegetable species in ORAC and TEAC antioxidant activity levels. Wide variations in antioxidant activity among the tested plants were also observed in our study.

Polyphenols and flavonols are the most common compounds in herbs having strong antioxidant activity [49]. Information on the antioxidant activity of a wide range of plants interests researchers, nutritionists, herb growers, consumers, and public health workers. Our results revealed a wide range of antioxidant activity among plants, indicating great potential benefits from diversifying diets in order to increase the consumption of antioxidants. Several plants in this study were high in antioxidant activity, including DM (dry), SO, ArP (fresh), and OC. These four plants have been part of East Asian diets for many years and have dual functions as food and medicine for the treating inflammation and gastric cancer [32,36,50]. In addition, the above mentioned antioxidant species are well adapted to tropics and subtropics, and can be utilized to develop functional foods as well as health promoting and pharmaceutical agents. Antioxidant activity values obtained by *in vitro* methods, including those in the present study, explain antioxidant functions more from a chemical perspective rather than in terms of biological mechanisms. Possible applications of the selected target plant extracts as food supplement for human health care are also under evaluation in our laboratory. Such plants merit further investigation to determine their specific antioxidants and biological functions.

4. Conclusions

Tested herbaceous plant extracts had strong antioxidant activity. OC, SO, DM (dry), and ArP (fresh) were rich in polyphenols and quercetin, and exhibited much higher antioxidant activity than the other plants tested. Significant and positive correlations among TEAC values and polyphenols and anthocyanidins were observed. ORAC values were generally higher and better correlated with antioxidant substances in comparison to TEAC and DPPH assays. The data in this study also add information to the flavonoid database and contribute to studies on health benefits from flavonoid intake, especially for populations consuming tropical and underutilized herbaceous plants. These results may benefit further *in vivo* studies to assess the therapeutic potential of phytochemicals as natural antioxidants.

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