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Antioxidant Capacity Connection with Phenolic and Flavonoid Content in Chinese Medicinal Herbs

Mengfei Li[1], Paul W. Pare[2], Jinlin Zhang[3], Tianlan Kang[4], Zhen Zhang[1], Delong Yang[1], Kepeng Wang[1] and Hua Xing[1*]

[1]*Gansu Provincial Key Lab of Aridland Crop Science / College of Life Science and Technology, Gansu Agricultural University, 730070 Lanzhou, P.R. China*

[2]*Department of Chemistry and Biochemistry, Texas Tech University, Lubbock, 79409 Texas, USA*

[3]*College of Pastoral Agriculture Science and Technology, Lanzhou University, 730000 Lanzhou, P.R. China*

[4]*Station of Industrial Crop Promotion of Gansu province, 730000 Lanzhou, P.R. China*

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Abstract: Traditional Chinese herbal medicines (TCHMs) have been used to treat diseases for thousands of years because of high therapeutic performance and low toxicity. To mine for new natural sources of antioxidants, 93 TCHMs were screened for activity, based on classical antioxidant capacity assays. Substantial differences in antioxidant capacity were coupled with phenolic and flavonoid content for each of the examined species. Species that exhibited both high antioxidant capacity and specialized-phytochemical content included: *Angelica dahurica*, *Atractylodes macrocephala*, *Paeonia lactiflora*, *Paeonia suffruticosa* and *Perilla frutescens.* These species have been identified as promising sources for natural antioxidants.

Keywords: Traditional Chinese herbal medicines; antioxidant capacity; total phenolic and flavonoid content.

1. Introduction

Reactive oxygen species (ROS) are chemically reactive molecules containing oxygen, which are formed as a natural byproduct of the normal metabolism of oxygen and have important roles in cell signaling and homeostasis, while under oxidative stress conditions, the levels of ROS can increase dramatically which can damage cellular proteins, lipids and DNA, leading to fatal lesions, such as aging, cancer, cardiovascular disease [1]. Antioxidants can terminate the oxidation chain reactions by removing free radical intermediates, and inhibit other oxidation reactions [2]. Although cell maintains complex systems of multiple types of antioxidants, such as glutathione, vitamin C and vitamin A, while the insufficient levels of antioxidants or inhibition of the antioxidant enzymes could cause oxidative stress [3].

* Corresponding author: E-Mail: xingh@gsau.edu.cn; Phone: 86-9317631742 Fax: 86-9317631875

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The exogenous natural antioxidants are widely used in clinic, and many studies have proved that natural antioxidants are low toxicity and non-carcinogenic in animal models [4]. Therefore, it is very important to find out new sources antioxidants from natural plants [5]. In China, TCHMs have been used to treat diseases for thousands of years because of their high therapeutic performance and low toxicity. Biological assays and phytochemical investigations have revealed that medicinal plants possess more potent antioxidant activity, phenolic and flavonoid compounds were major contributors to the antioxidant capacities [6]. Some previous studies have reported on screening natural antioxidants from about 300 medicinal plants by detecting the antioxidant capacity, total phenolic and flavonoid content [5,7,8]. In the 1977 *Encyclopedia of Traditional Chinese Meicinal Substances*, 5767 substances are identified as part of the traditional *materia medica*, and a typical practitioner may routinely use between 200 and 600 substances [9]. Thus, further researches on screening natural antioxidants from medicinal plants need to be conducted.

In this study, antioxidant capacities, total phenolic and flavonoid content in 93 TCHMs were evaluated using DPPH and FRAP assays, Folin–Ciocalteu and $NaNO_2$-$AlCl_3$-NaOH methods. The results would provide useful information and reference for prevention and treatment of diseases caused by oxidative stress in the future.

2. Materials and Methods

2.1. Plant Materials

The 93 TCHMs were collected from genuine regional locations in P. R. China. The species was identified by Prof. Yanling Qi (Gansu Provincial Academy of Agricultural Sciences, Lanzhou, P. R. China). The voucher specimens were deposited in the herbarium of College of Life Science and Technology, Gansu Agricultural University, Lanzhou, P. R. China. Herbarium numbers of voucher specimens are given in Table 1.

2.2. Reagents

DPPH (1, 1-Diphenyl-1-picrylhydrazyl), TPTZ (2, 4, 6-tris (2-pyridyl)-s-triazine), gallic acid and catechin were purchased from Sigma Chemical Company (St. Louis, MO, USA). Ethanol, methanol, HCl, $AlCl_3$, $FeCl_3 \cdot 6H_2O$, $FeSO_4 \cdot 7H_2O$, Folin-Ciocalteu reagent, Na_2CO_3, $NaNO_2$ and NaOH were purchased from Tianjin Guangfu Chemical Research Institute (Tianjin, P. R. China). All chemicals used in the test were of analytical grade.

2.3. Preparation of Extracts

The materials were cleaned and grinded to powder. Then the powder was weighted (2.00 g) and soaked in 70% aqueous ethanol (50.0 mL) for 72 h at room temperature and then filtered to recover the supernatant. The supernatant was concentrated using a rotary vacuum evaporator at 37 ºC, then the concentration was diluted to 5.0 mL with 15% aqueous ethanol. The dilution was stored in glass container at 4ºC for determination of antioxidant capacity, total phenolic and flavonoid content.

2.4. Antioxidant Capacity

The antioxidant capacity of the plant extract depends on, not only the compositions of the extract but also the test method [5]. Although there are numerous methods for determining the antioxidant capacity of soluble natural extracts and insoluble food components [10], no perfect system is available to help us know the “true” antioxidant capacity of a complex medium [11]. The DPPH and FRAP assays, are used by many researchers for rapid evaluation of antioxidant [12,13].

2.5. DPPH Radical Scavenging Assay

The free radical scavenging activity of DPPH was measured according to the description [14]. This is one of the few stable and commercially available organic nitrogen radical assays [15]. It is an electron transfer reaction. The initial electron transfer occurs very quickly, while the subsequent hydrogen transfer occurs more slowly and depends on the hydrogen-bond accepting solvent [16]. This reaction has been measured by the decoloration assay where DPPH has an absorption band at 515 nm which disappears upon reduction by an antiradical compound [17]. The specific steps are as follows. Briefly, 200 μL(10 mg/mL 15% aqueous ethanol) of the diluted extract was added with 3.80 mL of 10^{-4}mol/L DPPH methanol solution. Then the mixture was shaken and kept in dark for 30 min at room temperature. The decreased absorbance of DPPH solution was evaluated at 515 nm by a spectrophotometer. The capability to scavenge the DPPH radicals was calculated as follows:

DPPH scavenging activity (%) = $[(A_0\text{-}A)/A_0] \times 100$

Where "A_0"and "A" were the absorbance of DPPH without and with sample, respectively.

2.6. Ferric Reducing Antioxidant Power (FRAP) Assay

In the FRAP test, reductants (antioxidants) in the sample reduce ferric-tripyridyltriazine complex (Fe^{3+}- TPTZ), in stoichiometric excess, to a blue ferrous form (Fe^{2+}), with an increase in absorbance at 593 nm [17]. The specific steps were described by the literature [18]. Briefly, the working FRAP reagent was prepared *ex* tempore by mixing 10 volumes of 300 mmol/L acetate buffer, pH 3.6, with 10 mmol/L TPTZ in 40 mmol/L HCl, and 20 mmol/L $FeCl_3 \cdot 6H_2O$ at 10:1:1 (v/v/v). The 300 μL FRAP reagent and the 10 μL standard samples ($FeSO_4 \cdot 7H_2O$, 500 μmol) or test samples (10 mg/mL 15% aqueous ethanol) were added and mixed well. The reaction temperature was 37 °C and the absorbance readings were taken at 593 nm immediately and 4 min later using a spectrophotometer. The FRAP value of the test samples was calculated on the basis of 500 μM Fe^{2+} ($FeSO_4 \cdot 7H_2O$) as follows:

FRAP value (μmol Fe(II)/g)= (ΔA_{593} test sample /ΔA_{593} standard sample) × 500 (μmol Fe(II)/g)

Where ΔA_{593} was the absorbance of the sample minus the absorbance of the blank at the 4th minute.

2.7. Determination of Total Phenolic Content

The total phenolic content of the extracts was estimated using the Folin-Ciocalteu method with slight modification [19,20]. Briefly, 400 μL of extract was added with 2.00 mL of 10% Folin-Ciocalteu reagent and 1.60 mL of 7.5% Na_2CO_3 solution. Then the mixture was shaken for 5 min and then incubated at 37°C for 15 min, followed by incubation in the dark for 1 h. Absorbance was measured at 725 nm using a spectrophotometer. The standard calibration curves were daily prepared using gallic acid (GAE), the calibration equations C (GAE μg) = 34.48 A + 0.72 (R^2 =0.994). The total phenolic content was calculated as follows:

Total phenolic content (mg GAE/g DW) = $(C \times V_2) / (V_1 \times M \times 1000)$

Where C, V_1, V_2, A, *and* M represented total phenolic amount, sample test volume, extracts volume, sample absorbance, and materials dry weight (DW), respectively.

2.8. Determination of Total Flavonoid Content

The total flavonoid content of the extracts was determined using the $NaNO_2$-$AlCl_3$-NaOH method with slight modification [20,21]. Briefly, 400 μL of extract was added with 2.00 mL ddH_2O and 0.3mL of 5% $NaNO_2$. After 5 min, 0.3 ml of 10% $AlCl_3$ were added. After 1 min, 2.00 mL of 1.0 mol/L NaOH was added, and the solution was mixed with a vortex. Absorbance at 510 nm was measured against a blank with the spectrophotometer. The standard calibration curves were daily prepared using catechin (CE), the calibration equations C (CE μg) = 200 A—5.80 (R^2 = 0.996). The total flavonoid content was calculated as follows:

Total flavonoid content (mg CE/g DW) = $(C \times V_2) / (V_1 \times M \times 1000)$

Where C, V_1, V_2, A, and M represented total flavonoid amount, sample test volume, extracts volume, sample absorbance, and materials dry weight (DW), respectively.

2.9. Statistical Analysis

All tests were carried out in triplicate. The results were presented as the mean ± standard error of triplicate determinations. Correlation and regression analyses were performed using Excel and SPSS 11.5. Analysis of bivariate correlation (2-tailed) was used to evaluate the differences.

3. Results and Discussion

3.1. Antioxidant Capacity of the 93 TCHMs

The antioxidant capacity of the extract cannot be fully described with one single method [5,22]. A reliable antioxidant protocol requires the measurement of more than one property because most natural antioxidants are multifunctional. Therefore, it is essential to perform more than one type of antioxidant capacity measurement to take into account the various mechanisms of antioxidant action [23].

In this study, the antioxidant capacities displayed a large difference in both DPPH and FRAP assays in Table 1. The DPPH scavenging activities ranged from 23.85 % to 94.48 %, *Paeonia lactiflora* Pall had the highest level with 94.48 %, then *Paeonia suffruticosa* Andr (93.76 %) and *Angelica dahurica* Benth. et. Hook (93.65 %), but *Kadsura interior* had the lowest level with 23.85 %. For the FRAP assay, the FRAP values ranged from 53.67 to 3713.75 μmol Fe (II)/g, *Rhus chinensis* Mill had the highest values with 3713.75 μmol Fe(II)/g, then *Perilla frutescens* (L.) Britt (3577.21 μmol Fe(II)/g) and *Angelica dahurica* Benth. et. Hook (2451.98 μmol Fe(II)/g), but *Radix codonopsis* showed the lowest value with 53.67 μmol Fe(II)/g. It has been reported that there were large differences in the antioxidant capacities, such variations from 0.14 to 1844.85 μmol Fe(II)/g in 223 medicinal plants[5], from 0.24 to 2025.33 μmol Fe(II)/g in 40 medicinal plants [22], and 3.88 to 580.02 μmol Fe(II)/g in 56 medicinal plants [24]. These medicinal plants possessed high antioxidant capacities when compared with some fruits, vegetables, seeds and other medical plants and could be potential rich sources of natural antioxidants [25-28].

3.2. Total Phenolic and Flavonoid Content of the 93 TCHMs

As an important category of phytochemicals, phenolic compounds widely exist in plants and have been considered to be a major contributor to the antioxidant activity [29, 30]. Phytochemical investigations have revealed that there was a large difference among phenolic contents in medicinal plants with 0.19 to 101.33 mg GAE/g DW [5], 0.38 to 75.71 mg GAE/g DW [22], and 0.12 to 59.43 mg GAE/g DW [24]. As shown in Table 1, the total phenolic contents ranged from 15.06 to 62.35 mg GAE/g DW, *Perilla frutescens* (L.) Britt possessed the highest content with 62.35 mg GAE/g DW, then *Angelica dahurica* Benth. et. Hook (60.13 mg GAE/g DW) and *Atractylodes macrocephala* Koidz (58.87 mg GAE/g DW), but *Kadsura interior* showed the lowest content with 15.06 mg GAE/g DW. The total phenolic content of the 93 TCHMs was generally high when compared with some fruits, vegetables, seeds and other medical plants reported in the literature [25-28].

Flavonoids, as one kind of plant secondary metabolites, are not only vital function in plant growth and development, but also play an important role in free radical scavenging activity [31, 32]. As shown in Table 1, the total flavonoid content ranged from 0.68 to 13.85 mg CE/g DW, *Lonicera japonica* Thunb possessed the highest content with 13.85 mg CE/g DW, then *Citrus reticulata* (13.73 mg CE/g DW) and *Isatis tinctoria* (12.93 mg CE/g DW), but *Cynomorium songaricum* Rupr showed the lowest content with 0.68 mg CE/g DW. This indicated that flavonoids in medical plants might be essential phytochemical compounds in antioxidant capacity.

Table 1. Antioxidant capacities, total phenolic and flavonoid content in the 93 TCHMs

Voucher numbers of specimens	Species	Part of plant	DPPH scavenging activity (%)	FRAP values (μmol Fe(II)/g)	Phenolic content (mg GAE/g DW)	Flavonoid content (mg CE/g DW)
GAU-A-012R	*Achyranthes bidentata* Blume	Root	41.57±1.71	117.70±18.29	18.66±2.72	8.62±0.32
GAU-A-015S	*Agrimonia pilosa* Ldb	Stem and leaf	77.97±0.72	1182.67±58.05	32.93±0.49	6.22±0.03
GAU-A-018T	*Alisma plantago-aquatica* Linn	Tuber	91.69±0.88	1119.59±27.44	35.10±2.41	2.02±0.43
GAU-A-022B	*Allium macrostemon* Bunge	Bulbs	49.58±1.39	101.69±4.85	26.37±0.84	7.58±0.61
GAU-A-035F	*Amomum villosum*	Ripe fruit	83.33±0.52	1060.26±49.76	47.40±1.93	7.74±0.65
GAU-A-038S	*Andrographis paniculata* (Burm. f.) Nees	Stem and leaf	57.63±1.08	106.40±28.30	34.60±0.47	6.16±0.47
GAU-A-042R	*Anemarrhena asphodeloides* Bunge	Root	78.95±3.86	299.44±9.92	25.72±0.28	7.38±0.84
GAU-A-066R	*Angelica dahurica* Benth. et. Hook	Root	93.65±0.34	2451.98±98.87	60.13±4.77	7.71±1.17
GAU-A-067R	*Angelica sinensis*	Root	87.04±0.17	101.69±22.06	26.39±1.32	5.12±0.68
GAU-A-072F	*Areca catechu*	Ripe fruit	62.40±6.12	847.46±70.72	20.55±0.69	4.39±0.25
GAU-A-072S	*Asarum sieboldii* Miq	Stem and leaf	77.59±0.69	357.82±37.62	43.28±0.30	9.66±0.33
GAU-A-075R	*Asparagus cochinchinensis* (Lour.) Merr	Root	76.87±0.63	489.27±9.55	45.43±0.11	2.32±0.26
GAU-A-077R	*Aster tataricus* L. f	Root	87.11±0.77	1330.51±33.90	38.28±8.19	12.88±6.41
GAU-A-080R	*Atractylodes Lancea* (Thunb.) DC	Root	77.85±0.64	560.26±14.72	36.08±0.29	4.74±0.08
GAU-A-082R	*Atractylodes macrocephala* Koidz	Root	93.24±0.43	2214.69±58.37	58.87±4.05	2.08±0.25
GAU-B-054R	*Bupleurum chinense* DC	Root	86.28±0.41	278.72±42.87	46.32±2.53	9.04±0.51
GAU-C-004F	*Carthamus tinctorius* L	Flower	63.87±1.05	765.54±10.19	36.91±0.79	3.26±0.56
GAU-C-025F	*Chaenomeles sinensis* (Thouin) Koehne	Ripe fruit	47.96±3.81	1629.94±122.91	31.98±3.53	6.43±0.82
GAU-C-031R	*Cistanche deserticola* Ma	Root	78.23±2.75	157.25±23.01	33.60±0.51	3.44±0.28
GAU-C-034F	*Citrus aurantium* L	Ripe fruit	77.59±0.82	583.80±17.03	25.66±0.40	4.01±0.33
GAU-C-034F	*Citrus aurantium* L	Unripe fruit	87.07±0.63	351.22±85.00	20.80±0.07	8.91±0.49
GAU-C-037F	*Citrus reticulata*	Ripe fruit bark	88.66±0.41	435.97±37.93	53.90±0.43	13.73±1.02
GAU-C-041L	*Clematis chinensis*	Stem and leaf	90.21±0.07	177.02±49.44	38.23±4.79	2.91±0.02
GAU-C-045L	*Cocculus orbiculatus* (L.) DC	Leaf	91.65±0.24	419.02±33.70	35.80±0.38	10.62±1.15
GAU-C-047R	*Coptis chinensis* Franch	Root	30.31±0.62	505.65±26.95	23.91±0.23	5.00±0.63
GAU-C-049F	*Cornus officinalis* Sieb. et Zucc	Ripe fruit	88.47±0.43	970.81±65.79	38.01±6.46	5.67±0.62
GAU-C-051B	*Cortex Dictamni*	Root bark	73.89±2.31	141.24±51.78	25.85±0.38	2.72±0.03
GAU-C-053S	*Corydalis bungeana*	Stem and leaf	72.68±0.79	340.87±55.52	36.54±0.47	4.91±0.25
GAU-C-054F	*Crataegus pinnatifida* Bunge	Ripe fruit	82.28±0.51	1062.15±87.02	40.74±2.15	12.16±2.26
GAU-C-056R	*Curcuma aromatica* Salisb	Root	74.75±1.46	499.06±96.27	29.25±1.06	9.71±0.51
GAU-C-060S	*Cynomorium songaricum* Rupr	Stem and leaf	52.83±4.89	435.03±21.51	22.68±3.26	0.68±0.09
GAU-D-002R	*Davallia mariesii* Moore ex Bak	Root	85.90±0.43	957.63±96.67	30.11±1.76	10.25±0.04
GAU-D-012R	*Dendrobium nobile* Lindl	Root	87.19±0.52	177.97±53.89	44.30±3.43	4.95±0.31
GAU-D-017S	*Dichondra repens* Forst	Stem and leaf	87.64±0.11	505.65±7.47	37.04±3.98	9.01±0.58
GAU-D-018R	*Dioscorea opposita* Thunb	Root	74.57±2.03	1208.10±50.87	38.13±0.41	10.94±1.77
GAU-D-032R	*Dolomiaea souliei*	Root	89.91±1.01	338.04±37.88	38.52±3.92	11.16±0.40

Table 1 Continued..

GAU-E-028S	*Epimedium brevicornu* Maxim.	Stem and leaf	78.80±0.30	784.37±91.33	35.50±0.37	3.70±0.17
GAU-E-033B	*Eucommia ulmoides* Oliver	Bark	58.88±1.87	725.05±77.79	19.38±0.26	6.88±0.43
GAU-F-012F	*Fructus Arctii*	Ripe fruit	90.14±0.34	313.56±44.84	30.98±4.13	5.38±0.10
GAU-F-014S	*Fructus Kochiae* Scopariae	Stem and leaf	80.05±0.93	1201.51±142.66	52.17±0.47	2.78±0.28
GAU-F-015F	*Fructus Ligustri* Lucidi	Ripe fruit	56.01±1.34	765.73±64.25	22.02±3.71	5.79±0.52
GAU-G-009R	*Gentiana scabra* Bunge	Root	83.90±0.11	80.04±21.20	37.13±0.62	6.84±0.24
GAU-G-017Se	*Ginkgo*	Seed	72.98±3.13	832.39±34.52	33.60±0.41	5.42±0.32
GAU-G-023R	*Glycyrrhiza uralensis* Fisch.	Root	80.54±1.08	1176.08±58.05	41.36±0.99	6.07±0.58
GAU-H-007F	*Hemerocallis citrina* Baroni	Flower	84.96±1.21	1168.74±53.47	41.00±3.78	11.26±2.23
GAU-H-016R	*Heracleum hemsleyanum* Diels	Root	90.38±0.30	1236.91±98.68	42.52±5.16	7.00±4.77
GAU-H-022S	*Houttuynia cordata* Thunb	Stem and leaf	64.36±1.51	642.18±50.87	27.23±0.40	4.23±1.38
GAU-I-025R	*Isatis tinctoria*	Root	88.66±0.59	1353.11±73.61	47.87±1.27	12.93±0.79
GAU-K-002S	*Kadsura interior*	Stem	23.85±1.08	382.11±90.06	15.06±0.49	4.65±0.51
GAU-L-007R	*Ligusticum chuanxiong* Hort	Root	92.33±0.24	1480.23±67.86	20.89±6.78	7.89±0.57
GAU-L-019R	*Lobed Kudzuvine*	Root	37.26±3.24	62.15±15.73	15.16±0.58	3.37±0.76
GAU-L-020S	*Lobelia chinensis* Lour	Stem and leaf	82.99±0.71	447.83±29.40	56.50±0.58	2.27±0.20
GAU-L-031S	*Lonicera japonica* Thunb	Stem	89.87±0.17	1531.07±37.05	34.36±0.26	13.85±0.58
GAU-M-006B	*Magnolia officinalis* Rehd. et Wils	Bark	60.24±2.77	1271.19±109.11	46.84±0.58	8.85±0.75
GAU-M-016F	*Melia toosendan* Sieb. et Zucc	Ripe fruit	91.76±0.13	1424.67±53.02	39.79±0.36	3.13±0.06
GAU-M-024L	*Morus* alba L	Leaf	88.62±0.13	103.58±7.11	16.65±5.31	7.17±0.57
GAU-M-024F	*Morus* alba L	Ripe fruit	64.40±0.45	729.76±5.88	22.16±0.64	6.28±0.45
GAU-M-024R	*Morus* alba L	Root bark	87.64±0.23	352.17±35.32	28.22±0.62	4.25±0.22
GAU-N-024R	*Notopterygium incisum*	Root	85.68±0.33	327.68±18.52	46.88±7.48	11.09±1.31
GAU-P-003R	*Paeonia lactiflora* Pall	Root	94.48±0.24	1060.26±72.97	37.26±2.70	9.91±0.76
GAU-P-004R	*Paeonia suffruticosa* Andr	Seed	93.76±0.20	1718.46±16.27	37.99±1.28	8.70±0.26
GAU-P-005R	*Paeonia veitchii* Lynch	Root	91.84±0.11	1753.30±28.53	35.36±1.51	7.27±0.14
GAU-P-006F	*Perilla frutescens* (L.) Britt	Ripe fruit	92.48±0.26	3577.21±77.48	62.35±4.49	7.78±0.50
GAU-P-006S	*Perilla frutescens* (L.)Britt	Stem	80.67±0.17	1280.60±34.87	38.43±0.88	7.58±0.70
GAU-P-013B	*Phellodendron amurense* Rupr	Bark	81.48±0.80	479.28±49.60	42.50±3.69	8.05±0.85
GAU-P-015S	*Phryma leptostachya* L	Stem and leaf	63.27±0.20	1414.31±35.77	25.88±1.81	3.48±0.02
GAU-P-018S	*Pinellia ternata* (Thunb.) Breit	Stem and leaf	81.29±1.89	232.58±21.58	32.10±0.36	5.73±0.24
GAU-P-022R	*Platycodon grandiflorus* (Jacq.) A. DC	Root	88.78±0.20	205.27±21.20	44.42±0.77	4.27±0.20
GAU-P-027S	*Pogostemon cablin* (Blanco) Benth	Stem and leaf	89.64±0.17	919.59±8.63	42.51±2.51	6.57±0.11
GAU-P-029R	*Polygala tenuifolia* Willd	Root	78.76±0.98	302.26±12.95	49.96±0.71	3.88±0.33
GAU-P-030R	*Polygonatum odoratum* (Mill.) Druce	Root	60.39±4.98	72.50±5.55	31.74±1.86	4.59±0.18
GAU-P-031R	*Polygonatum sibiricum*	Root	66.10±2.28	728.81±13.37	26.21±0.29	5.07±0.33
GAU-P-032R	*Polygonum multiflorum* Thunb	Root	83.71±0.58	1350.28±80.68	53.75±0.46	5.97±0.22

Table 1 Continued..

GAU-P-034Sc	*Poria cocos* (Schw.) Wolf	Sclerotia	89.87±1.26	152.54±7.47	41.29±0.47	7.91±0.06
GAU-P-036S	*Prunella vulgaris*	Stem and leaf	91.23±0.24	733.52±55.52	51.34±3.08	3.00±0.29
GAU-P-038Se	*Psoralea corylifolia* Linn	Seed	74.49±0.39	841.81±15.30	43.86±2.91	3.52±0.24
GAU-P-040R	*Pyrrosia lingua* (Thunb.) Farwell	Root	91.12±0.07	228.81±35.28	49.37±2.19	2.56±0.27
GAU-R-001R	*Radix codonopsis*	Root	85.90±2.72	53.67±10.19	28.63±5.63	9.04±0.98
GAU-R-002R	*Radix Sophorae* flavescentis	Root	91.12±0.47	589.45±73.14	57.39±3.03	4.55±0.15
GAU-R-007R	*Rheum palmatum* L	Root	32.50±3.69	209.98±79.11	21.53±0.33	5.91±1.37
GAU-R-008L	*Rhus chinensis* Mill	Leaf	92.48±0.29	3713.75±103.27	29.72±3.50	5.68±0.52
GAU-R-009R	*Rubus idaeus*	Ripe fruit	40.93±4.69	787.19±92.26	22.43±1.11	5.64±0.33
GAU-S-002R	*Saposhnikovia divaricata* (Trucz.) Schischk	Root	91.01±0.26	305.08±85.07	33.30±4.84	11.67±0.65
GAU-S-011R	*Schisandra chinensis* (Turcz.) Baill	Ripe fruit	91.19±0.17	516.95±64.60	37.04±1.27	8.87±0.25
GAU-S-012R	*Scrophularia ningpoensis* Hemsl	Root	72.34 ±1.37	1008.85±41.16	30.00±1.14	6.80±0.38
GAU-S-018R	*Scutellaria baicalensis* Georgi	Root	76.15±2.77	1806.03±112.65	39.95±2.23	6.76±0.89
GAU-S-019S	*Scutellaria barbata* D. Don	Stem and leaf	62.93±3.26	1630.89±26.70	55.36±0.34	8.66±0.79
GAU-S-035R	*Stemona sessilifolia* (Miq.) Miq	Root	89.00±0.23	537.66±69.79	52.93±7.29	4.02±0.48
GAU-T-014R	*Terminalia chebula* Retz	Ripe fruit	80.99±0.58	601.32±34.08	26.05±4.95	2.50±0.38
GAU-T-017S	*Thlaspi arvense* Linn	Stem and leaf	80.08±0.46	460.45±57.13	41.58±1.68	2.95±0.38
GAU-T-021R	*Trichosanthes kirilowii* Maxim	Root	62.17±2.22	456.31±49.12	16.39±0.47	7.32±0.57
GAU-V-002Se	*Vaccaria segetalis*	Seed	67.46±1.42	746.14±7.62	37.06±4.11	8.42±0.83
GAU-W-011F	*Wisteria sinensis* (Sims) Sweet	Flower	82.77±0.57	400.19±46.62	41.55±0.46	10.12±0.80

The results were presented as the mean ± standard error of triplicate determinations.

3.3. Regression Analysis on the Relationship between Phytochemical Contents and Antioxidant Capacities

A simple linear regression analysis was used to analyze the correlation between the DPPH scavenging activities and FRAP values. As shown in Figure 1, a significant positive correlation (R^2=0.2350) between DPPH scavenging activities and FRAP values was obtained, which indicated that the components capable of scavenging free radicals were the same to some degree from the different extracts of the TCHMs. This was in good accordance with the reported literature that the antioxidant capacities obtained from DPPH assay were usually consistent with FRAP assay [33,34].

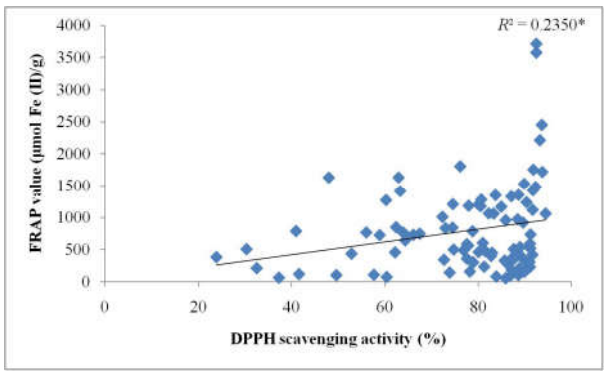

Figure 1. Relationship between DPPH scavenging activities and FRAP values in extracts from the 93 TCHMs.

The * indicated that the correlation was significant at $P < 0.05$.

As shown in Figure 2, there was an significant positive relationship of total phenolic content with DPPH scavenging activity and FRAP value, with correlation coefficient R^2=0.5410 (Figure 2A) and R^2=0.3280 (Figure 2B), respectively, which indicated that the phenolic compounds played important roles in antioxidant capacity. This result was in accordance with many previous researches reported in the literature [35, 36]. There was also a significant positive relationship of total flavonoid contents with DPPH scavenging activity, with the correlation coefficient R^2=0.2120 (Figure 2C), while that was with FRAP values didn't reach significant level with R^2=0.1170 (Figure 2D).

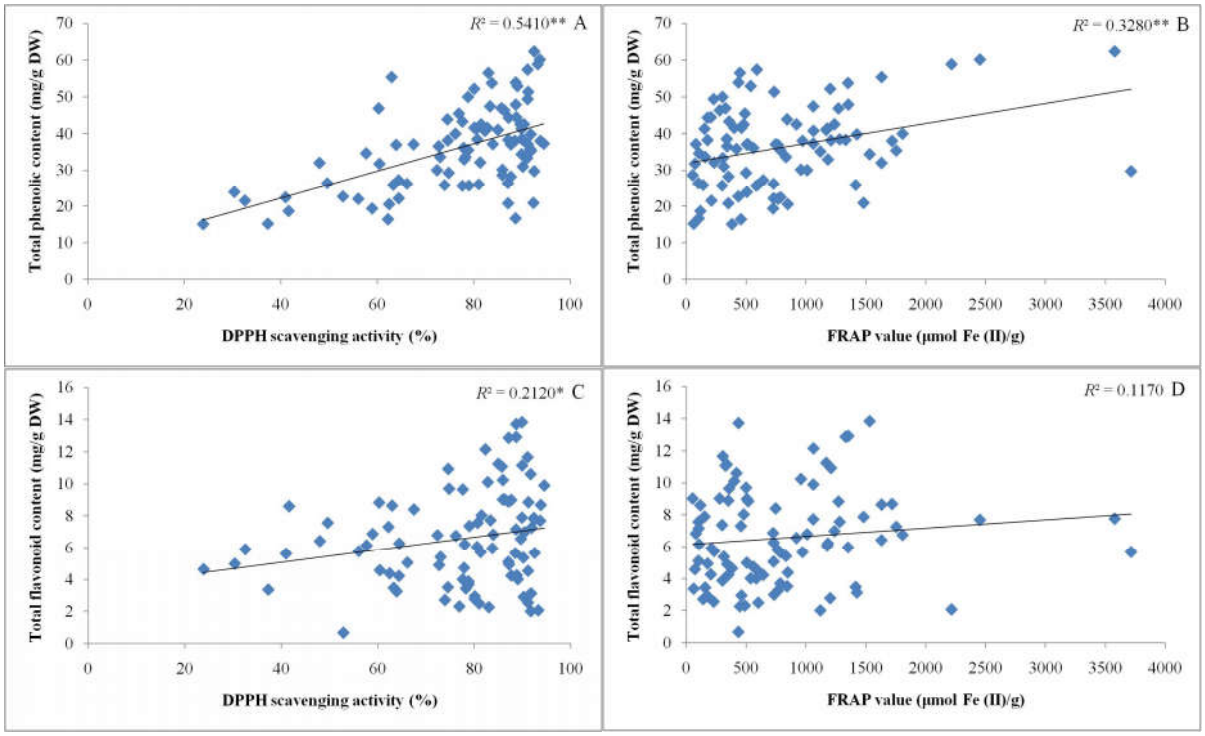

Figure 2. Relationship between phytochemical contents and antioxidant capacities in extracts from the 93 TCHMs

The * and ** indicated that the correlation was significant at $P < 0.05$ and $P < 0.01$ level, respectively.

3.4. Biological Activities of Top Five TCHMs

Based on the comprehensive consideration of DPPH scavenging activity, FRAP value, total phenolic and flavonoid content of the 93 TCHMs, five plants could be candidate for potential antioxidants as follows: *Angelica dahurica* Benth. et. Hook, *Atractylodes macrocephala* Koidz, *Paeonia lactiflora* Pall, *Paeonia suffruticosa* Andr, and *Perilla frutescens* (L.) Britt. The main biological activities and bioactive constituents were given in Table 2.

As a conclusion; biological assays and phytochemical investigations have revealed that the five plants possessed multiple biological activities, such as anti-tumor, anti-inflammatory, anti-viral, anti-aging and antioxidant activities, and contained many different compounds that might be directly related to antioxidant activities.

Table 2. Main biological activities and components of the top five TCHMs possessing high antioxidant capacities

Species	Main bioactivities	Main bioactive constituents	References
Angelica dahurica Benth. et. Hook	Anti-HIV-1, anti-microbial, anti-cancer, anti-tumour, anti-inflammatory, analgesic,hepatoprotective, nephroprotective	Imperatorin, oxypeucedanin, isoimperatorin, coumarins, byakangelicin, byakangelicol, bergapten, umbeliferone	[37-39]
Atractylodes macrocephala Koidz	Anti-tumor, anti-inflammatory, aromatase inhibitors, treatment of abdominal pain and gastroenterology diseases	Sesquiterpenes, acetylenic compounds, AtractylenolideI-III, caffeic acid, ferulic acid protocatechuic acid	[40-42]
Paeonia lactiflora Pall	Anti-influenza, anti-inflammatory, anti-hyperlipidemic, anti-hepatofibrosis, neuroprotective, immunomodulatory, treatment of rheumatoid arthritis, systemic lupus erythematosus, hepatitis, dysmenorrhea, muscle cramping and spasms	Paeoniflorin, albiflorin, oxypaeoniflorin, benzoylpaeoniflorin, oxybenzoyl-paeoniflorin, paeoniflorigenone,lactiflorin, galloylpaeoniflorin, paeonin, paeonolide, paeonol, paeonyglucosides	[43-45]
Paeonia suffruticosa Andr.	Anti-cancer, anti-diabetic, antioxidant activities, neuroprotectants, treatment of blood-heat and blood-stasis syndrome	Paeonol, paeonoside, paeonolide, paeoniflorin, apiopaeonoside, oxypaeoniflorin, benzoyl-paeoniflorin, benzoyl-oxypaeoniflorin, gallic acid	[46-48]
Perilla frutescens (L.) Britt (Ripe fruit)	Anxiolytic, anti-depressive, anti-inflammatory, anti-aging, anti-hyperlipidemia, anti-microbial, inhibitory activities against α-glucosidase and aldose reductase	Rosmarinic acid, caffeic acid, β-caryophyllene, 2-hexanoylfuran, β-farnesene, 1-cyclohexane-1-carboxaldehyde	[49-51]

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Competing Interests

Authors have declared that no competing interests exist.

ORCID

Mengfei Li: 0000-0003-3035-4427
Paul W. Pare: 0000-0003-1644-723X
Jinlin Zhang: 0000-0002-3562-920X
Tianlan Kang: 0000-0001-8528-787X
Zhen Zhang: 0000-0003-0519-1566
Delong Yang: 0000-0001-5370-1589
Kepeng Wang: 0000-0002-3293-7565
Hua Xing: 0000-0002-9699-1130

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