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Antioxidant and Anti-inflammatory Activities of Selected Medicinal Plants Containing Phenolic and Flavonoid Compounds

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ABSTRACT: The antioxidant, anti-inflammatory, and cytotoxic activities of water and ethanol extracts of 14 Chinese medicinal plants were investigated and also their total phenolics and flavonoid contents measured. The antioxidant activity was evaluated in a biological assay using *Saccharomyces cerevisiae*, whereas the radical scavenging activity was measured using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. Total phenolics and flavonoid contents were estimated by Folin−Ciocalteu and aluminum chloride methods, respectively. The anti-inflammatory activities of the plant extracts were determined by measuring the inhibition of production of nitric oxide (NO) and TNF-α in LPS and IFN-γ activated RAW 264.7 macrophages. Their cytotoxic activities against macrophages were determined by Alamar Blue assay. Four plants, namely, *Scutellaria baicalensis*, *Taxillus chinensis*, *Rheum officinale*, and *Sophora japonica*, showed significant antioxidant activity in both yeast model and also free radical scavenging methods. The ethanol extract of *S. japonica* showed highest levels of phenolics and flavonoids (91.33 GAE mg/g and 151.86 QE mg/g, respectively). A positive linear correlation between antioxidant activity and the total phenolics and flavonoid contents indicates that these compounds are likely to be the main antioxidants contributing to the observed activities. Five plant extracts (*S. baicalensis*, *T. chinensis*, *S. japonica*, *Mahonia fortunei*, and *Sophora flavescens*) exhibited significant anti-inflammatory activity by in vitro inhibition of the production of NO and TNF-α with low IC_{50} values. These findings suggest that some of the medicinal herbs studied in this paper are good sources of antioxidants.

KEYWORDS: medicinal plants, antioxidant activity, anti-inflammatory, phenolics, flavonoids

INTRODUCTION

Free radicals and reactive oxygen species (ROS) are constantly produced as byproducts in the human body during cell metabolism. These harmful byproducts, if not eliminated immediately, can cause oxidative damage to functional macromolecules such as DNA, proteins, and lipids.[1,2] This increases the chance of occurrence of age-related disorders, cancer, atherosclerosis, neurodegenerative diseases, and inflammation.[2,3] Consumption of antioxidants, through diet and supplements, is expected to remove ROS from the living system and provide health benefits. Several studies demonstrated that medicinal plants are a rich source of antioxidant compounds such as phenolics, flavonoids, quinones, vitamins, coumarins, and alkaloids, which can decrease the incidence of oxidative stress and associated diseases.[3] Recent studies demonstrated that plant phenolic compounds and flavonoids act as reducing agents (either by donating hydrogen atom and/or by quenching singlet oxygen), which explains their antioxidant activities.[4]

The literature strongly suggests that plant polyphenols (especially phenolics and flavoinoids) inhibit the inflammation process by regulating the production of pro-inflammatory molecules.[5] Pro-inflammatory molecules such as cytokine (TNF-α), leukocyte adhesion, and nitric oxide (NO) produced during inflammatory reactions have been shown to play a crucial role in immune-inflammatory response.[6,7] Excess production of NO and TNF-α is known to cause host cell death and inflammatory tissue damage.[7,8] It is also important to note that NO reacts with free radicals, such as superoxides, to produce highly damaging peroxynitrates, which can oxidize low-density lipoproteins that lead to irreversible damage to cell membranes.[7,8] Hence, inhibition of production of such pro-inflammatory molecules (NO and TNF-α) is expected to have therapeutic value against inflammatory diseases.

The free radical scavenging activity (antioxidant activity) has been evaluated using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method, which is regarded as an easy, reliable, sensitive, and rapid method as compared with other antiradical methods.[9] A review by Moon and Shibamoto also reported that 90% of antioxidant activity measurements are conducted by this method. In vitro antioxidant activities of the plants were evaluated on the basis of their ability to inhibit H_2O_2 induced oxidative stress in yeast cells (*Saccharomyces cervisiae*). This method has been employed for in vivo antioxidant studies as yeast is the ideal model organism for monitoring the biological mechanism.[10,11] The anti-inflammatory properties of selected plants were evaluated on the basis of inhibitory ability against the production of NO and TNF-α using the Griess reagent method and sandwich ELISA, respectively.

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Table 1. Chinese Medicinal Herbs Used in This Study

plant	plant name	family name	Chinese name	herb parts used	medicinal use	ref
1	*Sarcandra glabre* (Thunb.) Nakai	Chloranthaceae	Zhong jie feng	whole plant	antitumor and antibacterial	27
2	*Codonopsis pilosula* (Franch.) Nannf.	Campanulaceae	Dang shen	root	immunomodulatory activity	28
3	*Curcuma aromatica* Salisb	Zingiberaceae	Yu jin	root	anti-inflammatory, antioxidant, and anticarcinogenic	29
4	*Scutellaria baicalensis* Georgi.	Labiatae	Huang qin	root	anti-inflammatory and antitumor	30
5	*Taxillus chinensis* (DC) Danser	Loranthaceae	Sang ji sheng	stem and branch	obesity	31
6	*Eucommia ulmoides* Oliver	Eucomiaceae Engler	Du zhong	bark	anticancer and antifungal	32
7	*Atractylodes macrocephala* Koidz.	Compositae	Bai zhu	rhizome	anti-inflammatory activity	33
8	*Rheum officinale* Bail	Polygonaceae	Da huang	rhizome	digestive system diseases, treatment of various hemorrhages, and trauma	22
9	*Sophora japonica* (L.) Schott.	Fabaceae	Huai hua	flower	anti-inflammatory activity	34
10	*Polygonum cuspidatum* Sieb. et Zucc	Polygonacae	Hu Zhang	root and rhizome	antioxidant activty and anti-inflammatory activity	35
11	*Saposhnikovia divaricata* (Turcz.) Schischk	Apiaceae (alt. Umbelliferae)	Fang feng	root	anti-inflammatory	36
12	*Mahonia fortunei* (Lindl.) Fedde	Berberidaceae	Shi da gong lao	leaf	antibacterial and antifungal	37
13	*Sophora flavescens* Ait.	Fabaceae	Ku shen	root	antimicrobial	38
14	*Pinellia ternate* (Thunb.) Breit.	Araceae	Ban xia	rhizome	antimicrobial and anti-inflammatory	39

The toxicity effects of the plant extracts were evaluated using the Alamar Blue assay.

The approaches for selecting plants to be tested for their activity vary from random selection to more guided selection strategies such as the ethnopharmacological approach[12,13] and the experience derived from traditional practice.[14,15] For the study reported here, 14 medicinal plants (Table 1) have been carefully selected on the basis of ethnopharmacological importance[13] and traditional practice.[12,14] The main aim of the current study is to discover the plants that are good sources of natural antioxidants. Such plants will be useful for isolating newer antioxidants.

MATERIALS AND METHODS

Plant Materials. The dried plant materials were obtained from Beijing Tong Ren Tang Chinese Herbal Medicine shop, Sydney, Australia. A voucher specimen of each plant has been deposited in the laboratory. The scientific names and family names are given in Table 1. The plant materials were ground to a fine powder in a grinder before extraction.

Chemicals and Reagents. Gallic acid, quercetin, DPPH, dimethyl sulfoxide (DMSO), sodium carbonate, aluminum chloride ($AlCl_3$), sodium nitrate ($NaNO_2$), sodium hydroxide (NaOH), hydrogen peroxide (H_2O_2), Folin−Ciocalteu (F-C) reagent, ascorbic acid, 95% ethanol, bovine serum albumin (BSA), lipopolysaccharide (LPS: *Escherichia coli* serotype 0127:B8), *N*-(1,1-naphthyl)ethylenediamine dihydrochloride, penicillin G sodium benzyl, resazurin sodium 10%, streptomycin, sulfanilamide, tetramethylbenzidine (TMB), and Trypan Blue were purchased from Sigma (Australia) and Lomb Scientific Pty Ltd. (Australia). Antibiotics, Dulbecco's modified eagle's medium (DMEM), fetal bovine serum (FBS), and glutamine were purchased from GIBCO. Interferon-γ (murine) and tumor necrosis factor-α (TNF-α) enzyme-linked immunosorbent assay (ELISA) kits were purchased from Peprotech.

Preparation of the Water Extracts. Approximately 3 g of each ground plant material was autoclaved with 45 mL of deionized water (DIW) at 121 °C for 1 h. The extracted samples were centrifuged at 10447*g* for 20 min), the supernatant was transferred into a 50 mL volumetric flask, and the volume was adjusted to 50 mL. The samples were stored at −20 °C until analysis.

Ethanol Extracts. Ground samples (3 gms) were extracted with 45 mL of 95% ethanol on a water bath at 70 °C for 6 h. The extracted samples were centrifuged, and the supernatant was transferred into a 50 mL volumetric flask and its volume and adjusted to 50 mL with 95% ethanol. The samples were stored at −4 °C until analysis. All water and ethanol extracts were filtered before analysis.

Determination of Total Phenolic Compounds. The total phenolics content was determined by F-C colorimetric method.[16] Briefly, 50 μL of sample and 50 μL of F-C reagent were pipetted into an eppendorf tube. The contents were vortexed for 10 s and then left at room temperature for 2 min. After 2 min, 500 μL of 5% (w/v) sodium carbonate solution was added to stop the reaction, and then 400 μL of distilled water was added to make up to 1 mL. The vortexed reaction mixture was heated in a water bath at 45 °C for 30 min and then cooled rapidly in an ice bath. Absorbance was measured at 760 nm. Gallic acid concentrations ranging from 0 to 300 μg/mL were prepared, and the calibration curve was obtained using a linear fit (r^2 = 0.9961). The samples were analyzed in duplicate.

Determination of Total Flavonoids. Total flavonoids were estimated according to the aluminum chloride method.[17] Briefly, 0.5 mL of each sample and 300 μL of $NaNO_2$ (1:20 w/v) were pipetted into a test tube. The contents were vortexed for 10 s and left at room temperature for 5 min. Into the mixture were then added 300 μL of $AlCl_3$ (1:10 w/v), 2 mL of 1 M NaOH, and 1.9 mL of distilled water. After 10 s of vortexing, the absorbance for each sample was measured at 510 nm. Quercetin concentrations ranging from 0 to 1200 μg/mL were prepared, and the standard calibration curve was obtained using a linear fit (r^2 = 0.9980). The samples were analyzed in duplicate.

Free Radical DPPH Scavenging Assay. The DPPH radical scavenging assay was carried out by using the Blois method.[18] Each plant extract (50 μL) (water or ethanol) was added to a 150 μL of 62.5 μM DPPH. After 30 min of incubation, the absorbance of the reaction mixtures was measured at 492 nm using a microplate reader (Multiskan EX, Thermo Electron, USA). Ascorbate (vitamin C), an antioxidant, was used as a positive control. A standard curve was included for each plate with a series of ascorbate concentrations (0, 10, 20, 40, 60, 80, 100, 200, 400, and 1000 μM). The free radical reduction capacity for each herbal extract was calculated as the ascorbate equivalent against the ascorbate standard curve (r^2 = 0.9924).

Assay for Screening Scavenging Activity in a 96-Well Microplate by Using *S. cerevisiae*. The antioxidant capacities of the herbal extracts were also measured using a *S. cerevisiae*-based high throughput assay. *S. cerevisiae* BY4743 was cultured overnight in a 50 mL volume by inoculation of a single colony. The culture was then diluted to an optical density at 600 nm (OD_{600}) of 0.2 in media, and 180 μL of each strain was added into a well in a 96-well microtiter plate, where 10 μL per well of each herbal extract was also added in duplicate. Ten microliters of H_2O_2 was added to a final concentration of 4 mM. The initial OD_{600} reading was taken using a microplate reader (Multiskan EX, Thermo Electron, USA), and the plates were then incubated in a 30 °C incubator with shaking at 750 rpm. Yeast growth was monitored by reading OD_{600} at the end of 20 h. Ascorbic acid was used as a positive control.[19] The net growth of H_2O_2-induced yeast cells after the treatment of selected plant extracts was measured using the equation

$$P_{\text{yeastgrowth}} = \left(\frac{P_{\text{sample}} - P_{\text{control}}}{P_{\text{control}}}\right) \times 100$$

where $P_{\text{yeast growth}}$ = net growth of H_2O_2-induced yeast cells after treatment with plant extracts, P_{sample} = observed optical density of yeast cells with the treatment of plant extracts, and P_{control} = observed optical density of yeast cells with the treatment of negative control (H_2O_2).

Maintenance, Preparation, and Activation of RAW 264.7 Macrophages. RAW 264.7 macrophages were grown in 175 cm^2 flasks on DMEM containing 5% FBS that was supplemented with antibiotics (1%) and glutamine (1%). The cell line was maintained in 5% CO_2 at 37 °C, with media being replaced every 3−4 days. Once cells had grown to confluence in the culture flask, they were removed using a rubber policeman, as opposed to using trypsin, which can remove membrane-bound receptors such as RAGE.[20] Cell suspension was concentrated by centrifugation for 3 min at 900 rpm and resuspension in a small volume of fresh DMEM (with 1% antibiotics and 5% FBS). Cell densities were estimated using a Neubauer counting chamber. Cell concentration is adjusted with DMEM (with 1% antibiotics and 5% FBS) to obtain 75000 cells/well when 100 μL cell suspension was dispensed into the 60 inner wells of 96-well plates. Sterile distilled water was added to the outer row of wells and incubated at 37 °C and 5% CO_2 for 12 h. From each well conditioned medium was replaced with fresh serum-free medium. For assays with extracts, a 50 μL volume of the dilutions (in water) was added an hour prior to addition of activator. Due to the often inconsistent nature of LPS at activating cells, a combination of 25 μg/mL LPS and 10 U/mL IFN-γ, both in DMEM, was used for activation. Usually a maximum dose of the extracts used was 2.5 mg/mL, and a minimum of 6 doses were made by serial dilution. Then the cells were incubated for 24 h at 37 °C and 5% CO_2. Cells with media alone were used as negative control and activated cells as positive control.

Determination of Nitric Oxide Production by Griess Assay. Nitric oxide is determined by Griess reagent quantification of nitrite, one of its stable reaction products. Griess reagent is freshly made up of equal volumes of 1% sulfanilamide and 0.1% naphthylethylenediamine in 5% HCl. In the presence of nitrite this reagent forms a violet color. From each well 70 μL of supernatant was transferred to a fresh 96-well plate and mixed with 70 μL of Griess reagent, and the color produced was measured at 540 nm. The remaining supernatant that was removed from each well was used for TNF-α assay using a commercial sandwich ELISA.

Determination of Cell Viability by Alamar Blue Assay. The Alamar Blue assay is a colorimetric assay involving the cellular reduction of resazurin to resorufin. One hundred microliters of Alamar Blue solution (10% Alamar Blue (Resazurin) in DMEM medium) was added to each well and incubated at 37 °C for 1−2 h. Fluorescence was measured (excitation at 545 nm and emission at 595 nm) and expressed as a percentage of that in control cells after background fluorescence was subtracted.

TNF-α Determination by ELISA. Sandwich ELISA was used according to the manufacturer's manual (Peprotech) to determine TNF-α concentration. Capture antibody was used at a concentration of 0.5 μg/mL in PBS (1.9 mM NaH_2PO_4, 8.1 mM Na_2HPO_4, 154 mM NaCl; pH 7.4). Serial dilutions of TNF-α standard from 0 to 1000 pg/mL in diluent (0.05% Tween-20, 0.1% BSA in PBS) were used as internal standard. TNF-α was detected with a biotinylated second antibody and an avidin peroxidase conjugate with TMB as detection reagent. The color development was monitored at 655 nm, with readings taken after every 5 min. After 25 min, the reaction was stopped using 0.5 M sulfuric acid, and the absorbance was measured at 450 nm.

Data Presentation and Analysis. As the experiments were done in duplicate, the results were expressed as the mean ± standard deviation. In addition, linear relationships and significance tests of these data sets were also conducted. GraphPad Prism 5.01 was used for growth curve analysis in dose-dependent experiments and to determine the IC_{50} values for NO and TNF-α inhibition.

RESULTS AND DISCUSSION

Total Phenolics and Flavonoids Contents in the Selected Plants. The total phenolics and flavonoid contents of the selected 14 herbs were measured using F-C reagent and aluminum chloride methods, respectively. These results obtained for water and ethanol extracts of the plants are presented in Table 2. As can be seen from the table, significant phenolics content was observed in water extracts of *S. baicalensis* (60.52 GAE mg/g) and *R. officinale* (33.18 GAE mg/g). Moderate levels of phenolics content were found in water extracts of *T. chinensis*, *S. japonicas*, and *M. fortunei* (21.46, 27.84, and 28.6 GAE mg/g, respectively). The highest phenolics content was observed in the ethanol extracts of *S. japonica* (91.33 GAE mg/g) followed by *P. cuspidatum* (49.07 GAE mg/g), *S. baicalensis* (46.85 GAE mg/g), *R. officinale* (42.77 GAE mg/g), and *T. chinensis* (26.54 GAE mg/g).

The mean values of total flavonoids content varied from 0.73 to 78.52 QE mg/g, whereas in ethanol extracts it ranged from 1.05 to 151.86 QE mg/g. Among all of the water extracts, the highest flavonoid contents were found in *T. chinensis* (78.52 QE mg/g), *S. japonica* (66.76 QE mg/g), *M. fortunei* (59.13 QE mg/g), and *R. officinale* (54.57 QE mg/g). In addition to these plants, moderate levels of total flavonoids content were also found in *Sarcandra glabre*, *S. baicalensis*, and *P. cuspidatum*. The ethanol extracts of *S. japonica* (151.86 QE mg/g), *P. cuspidatum* (84.73 QE mg/g), *R. officinale* (98.51 QE mg/g), *T. chinensis* (64.64 QE mg/g), and *S. baicalensis* (58.46 QE mg/g) showed significantly high flavonoid contents. As can be seen from Table 2, the phenolics/flavonoid contents varied among all selected plants and in different extracts.

Antioxidant Activities of Selected Plant Extracts. The antioxidant activity of the selected medicinal herbs was evaluated by using DPPH free radical scavenging method and also yeast (*S. cervicea*) model.

The results of free radical scavenging capacity of the selected herbs are presented in Table 2. The scavenging capacity of water extracts ranged from 19.9 μM to 202.4 μM ascorbate equiv/g while for ethanol extracts the range is from 8.14 to 111.36 μM ascorbate equiv/g. High scavenging capacity was shown by water extracts of *S. baicalensis*, *R. officinale*, *S. japonica*, and *Polygonum cuspidatum* (Table 2). Among all the ethanol extracts *S. japonica*, *S. baicalensis*, *R. officinale*, and *P. cuspidatum* exhibited good scavenging activity (Table 2). *S. japonica* and *S. baicalensis* showed significant scavenging activity in both water and ethanol extracts.

Table 2. Antioxidant Activities of Selected Chinese Medicinal Herbs along with Their Total Phenolics and Flavonoid Contents

	DPPH scavenging activity[a] (ascorbate equivalent μM)		% yeast cell growth[b]	phenolics content[c] (GAE mg/g)		flavonoid content[c] (QE mg/g)	
plant	water extracts	ethanol extracts	water extracts	water extracts	ethanol extracts	water extracts	ethanol extracts
1	126.9 ± 9.19	68.5 ± 0.00	7.15	11.4 ± 2.73	6.64 ± 0.78	32.37 ± 3.91	24.78 ± 5.87
2	40.9 ± 2.12	8.14 ± 0.51	8.45	5.54 ± 1.36	3.78 ± 3.79	4.09 ± 0.98	12.91 ± 0.98
3	42.9 ± 3.54	30.29 ± 2.53	3.23	2.04 ± 0.27	0.82 ± 0.65	0.73 ± 0.00	2.46 ± 0.00
4	201.9 ± 0.71	107.43 ± 1.52	11.76	60.52 ± 0.96	46.85 ± 3.92	45.47 ± 5.87	58.46 ± 4.89
5	152.4 ± 0.00	91.36 ± 0.00	15.85	21.46 ± 0.41	26.54 ± 0.65	78.52 ± 0.00	64.64 ± 0.00
6	121.9 ± 0.71	57.07 ± 4.04	14.81	5.28 ± 0.00	6.84 ± 0.00	6.32 ± 0.98	29.03 ± 0.00
7	35.9 ± 4.95	49.21 ± 1.01	16.03	2.56 ± 2.46	2.05 ± 4.28	4.39 ± 1.96	10.99 ± 2.93
8	202.4 ± 0	98.14 ± 1.52	13.07	33.18 ± 2.59	42.77 ± 1.31	54.57 ± 0.98	98.51 ± 0.00
9	196.9 ± 0.71	111.36 ± 1.01	15.42	27.84 ± 3.28	91.33 ± 0.00	66.76 ± 1.96	151.86 ± 0.00
10	189.4 ± 4.24	91.71 ± 0.51	3.41	17.14 ± 3.28	49.07 ± 4.05	47.61 ± 0.00	84.73 ± 0.00
11	53.9 ± 0.71	20.29 ± 2.53	18.72	7.07 ± 0.27	6.03 ± 0.44	3.39 ± 1.96	40.59 ± 2.93
12	171.9 ± 3.54	84.57 ± 0.51	5.03	28.6 ± 4.37	9.82 ± 0.3	59.13 ± 0.98	40.32 ± 2.93
13	19.9 ± 2.12	35.64 ± 1.01	18.72	7.35 ± 0.96	10.05 ± 0.44	2.18 ± 0.00	9.01 ± 2.93
14	25.4 ± 2.83	32.79 ± 1.01	5.84	1.26 ± 0.14	1.09 ± 1.57	1.33 ± 0.98	1.05 ± 2.93

[a] DPPH free radical scavenging activity was measured in terms of equivalent of ascorbate (μM). [b] Yeast oxidative stress was measure on the basis of survival of yeast cells (yeast growth) after treatment with H2O2. [c] Total phenolics and flavonoid contents were expressed in gallic acid equivalent (GAE mg/g) and quercetin equivalent (QE equiv/mg), respectively.

Table 3. Anti-inflammatory Activities of the Selected Plants

	IC_{50} for inhibition of NO production		IC_{50} for inhibition of TNF-α production	
plant	(mg/mL)	cell viability[a] (% of cell survival)	(mg/mL)	cell viability[a] (% of cell survival)
1	0.11 ± 0.02	91 ± 8.8	0.4 ± 0.15	64.2 ± 1.6
2	0.62 ± 0.09	90.3 ± 10.7	>2.5	NA[b]
3	0.46 ± 0.12	94.7 ± 0.5	>2.5	NA
4	0.04 ± 0	91.8 ± 4.6	0.18 ± 0.05	73.6 ± 0.6
5	0.05 ± 0	87 ± 0	0.14 ± 0.05	63.45 ± 7.7
6	0.07 ± 0	95.7 ± 0	0.8 ± 0.2	86.7 ± 0.5
7	1.09 ± 0.2	104 ± 5.7	>2.5	NA
8	0.15 ± 0.08	99.5 ± 2.2	0.58 ± 0.24	65.7 ± 3.6
9	0.06 ± 0.02	91 ± 2.5	0.18 ± 0.08	78.7 ± 0.5
10	0.12 ± 0.04	95.4 ± 2.6	0.55 ± 0.12	56.9 ± 5.7
11	0.42 ± 0.36	104.7 ± 7.6	>2.5	NA
12	0.09 ± 0	95 ± 0	0.17 ± 0	93.6 ± 1.1
13	0.13 ± 0.08	89.6 ± 6.7	0.08	94.6 ± 6.7
14	1.05 ± 0.05	108.5 ± 9.2	>2.5	NA

[a] Cell viability was measured at appropriate IC_{50} values corresponding to the inhibition of NO and TNF-α. [b] NA, not analyzed.

Antioxidant activities of water extracts of the selected plants measured by yeast model are presented in Table 2. These results revealed that the extracts of *S. flavescens* (18.72%), *Saposhnikovia divaricata* (18.72%), *Atracylodes macrocephala* (16.03%), *S. japonica* (15.42%), *T. chinensis* (15.85%), *Eucommia ulmoides* (14.81%), *R. officinale* (13.07%) and *S. baicalensis* (11.76%) have high activity. It is interesting to note that the plants *Scutellaria baicalensis*, *Taxillus chinensis*, and *Sophora japonica* showed significant activity in both the DPPH method and yeast model.

Anti-inflammatory Activity of Plant Extracts. The anti-inflammatory activities of plants as measured using the RAW 264.7 macrophage model are presented in Table 3.

As can be seen from these results (Table 3), the extracts of *S. baicalensis*, *T. chinensis*, , *E. ulmoides*, *S. japonica*, and *M. fortunei* have down-regulated NO production with IC_{50} values of <0.1 mg/mL without significantly affecting cell viability (>85%). As can be seen from Table 3, the inhibition efficiency of plant extracts with respect to NO production was superior when compared to that of TNF-α production. Among all of the plants, *M. fortunei* (IC_{50} = 0.17 mg/mL), *S. baicalensis* (IC_{50} = 0.18 mg/mL), *T. chinensis* (IC_{50} = 0.14 mg/mL), *S. japonica* (IC_{50} = 0.18 mg/mL), and *S. flavescens* (IC_{50} = 0.08 mg/mL) have shown significant inhibition of TNF-α production with good cell viabilities.

It is important to note that the water extracts of three plants, namely, *S. baicalensis*, *T. chinensis*, and *P. cuspidatum*, have significant anti-inflammatory activity in terms of inhibiting the production of both NO and TNF-α with significant cell viability (Table 3).

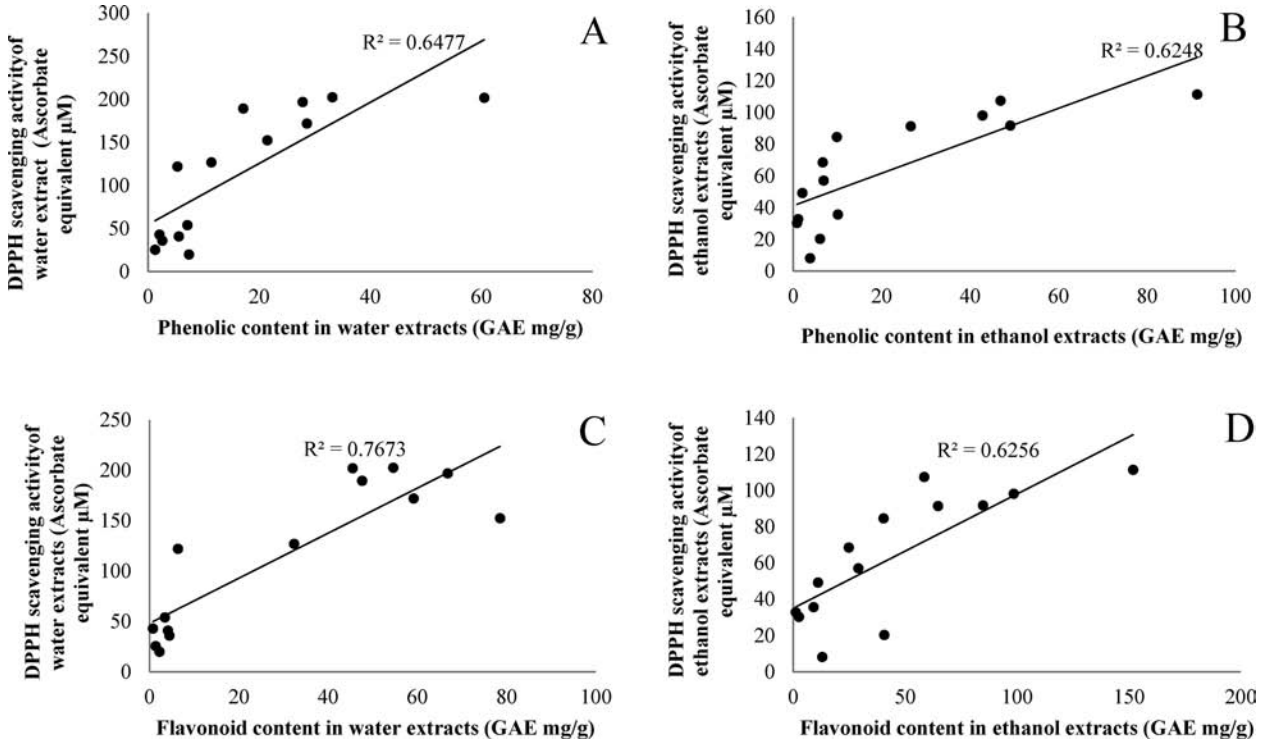

Figure 1. Correlation between DPPH free radical scavenging activity and the total phenolics content in (A) water extracts and (C) ethanol extracts and between DPPH free radical scavenging activity and total flavonoid content in (B) water extracts (D) and ethanol extracts.

Correlation of Antioxidant Activities of Selected Plants in Terms of Their Total Phenolics and Flavonoid Contents. To understand the antioxidant activities of the selected medicinal herbs in terms of their antioxidant content (total phenolics and flavonoids), correlation plots were developed (Figure 1). In water extracts the DPPH scavenging activity showed significant correlation with total phenolics content (Figure 1A, 0.647, $p < 0.05$) and also with the total flvavoid content (Figure 1C, 0.7673, $p < 0.05$). The correlation of antioxidant properties to their total phenolics (Figure 1B, 0.6248, $p < 0.05$) and flavonoid (Figure 1D, 0.6256 $p < 0.05$) contents was also significant in ethanol extracts.

The results presented above are in agreement with the fact that the total phenolics and flavonoid contents are major contributors to the antioxidant activity of herbal medicine, which is in accordance with the literature.[21−24] The herbs *S. baicalensis*, *T. chinensis*, *R. officinale*, *S. japonica*, and *P. cuspidatum* studied here showed high DPPH scavenging activity and also have high total phenolics and flavonoid contents (Table 2). It is also interesting to note that several flavonoid and flavonoid glycosides have been isolated from *S. japonicas*.[23] Results reported in the literature[24] confirm that *P. cuspidatum* possesses high phenolics and flavonoid contents.

Although the correlations of antioxidant activity with total phenolics and flavonoids presented in this paper are significant (Figure 1), some of the herbs significantly deviated from the linear correlation. For instance, *Eucommia ulmoides* and *Sarcandra glabre* have been found to have high antioxidant activity but low levels of phenolics/flavonoid contents (Table 2). It is therefore reasonable to conclude that in addition to phenolics and flavonoids there are other antioxidants that may be present in medicinal herbs and contribute to their antioxidant activities.

Correlation of Anti-inflammatory Activities of Selected Plants in Terms of Their Total Phenolics and Flavonoid Contents. It is interesting to note from the results that there is a fair degree of correlation between the anti-inflammatory activities and total phenolics and flavonoid contents. For instance, *Scutellaria baicalensis*, *Taxillus chinensis*, and *Sophora japonica* inhibited the production of NO and TNF-α (low IC_{50} values) and also have significant levels of total phenolics and flavonoid contents. Other plants such as *Codonopsis pilosula*, *Curcuma aromatic*, *Atractylodes macrocephala*, *Saposhnikovia divaricata* and *Pinellia ternate*, were found to contain low levels of phenolics/flavonoid contents and also lower antioxidant activity (Table 2).

These results are in agreement with the previous studies that phenolics and flavonoids influence anti-inflammatory activities.[25] Recently, several compounds that have been isolated from medicinal herbs showed strong anti-inflammatory activities. For instance, phenolic compounds, namely, baicalein, oroxylin A, and wogonin, isolated from *S. baicalensis* significantly inhibited the production of NO.[26] The literature also supports that isoflavone glycosides isolated from *S. japonica* show significant inhibition of chemical modulators formed during inflammatory response.

On the other hand, the anti-inflammatory activities of some of the plants are not consistent with the total phenolics and flavonoid contents. For instance, *S. flavescens* is found to have significant anti-inflammatory activities (inhibition of production of both NO and TNF-α), but this plant contains significantly low levels of phenolics and flavonoids. Hence, it is concluded that chemical constituents other than phenolics and flavonoids may also be responsible for the anti-inflammatory properties of such plants.

From the results presented above, it is clear that some of the plants studied (*S. baicalensis*, *T. chinensis*, *S. japonica*, and *M. fortunei*) are good sources of antioxidant/anti-inflammatory compounds. Currently, the isolation of bioactive compounds from *T. chinensis* and other plants is underway in our laboratory.

Conclusion. In this study, total phenolics and flavonoid contents and antioxidant and anti-inflammatory activities of 14 selected medicinal herbs were evaluated. The correlation between polyphenolics content and antioxidant activities is in agreement with previous investigations that the polyphenolic compounds contributed significantly to the antioxidant activities of the medicinal herbs.[2,3] A similar correlation, although less prominent, was also observed between polyphenolics content and anti-inflammatory activities. Among all of the plants studied, *S. baicalensis*, *T. chinensis*, *S. japonica*, and *M. fortunei* exhibited high antioxidant and anti-inflammatory activities. Isolation of bioactive compounds from *T. chinensis* and other plants is currently underway in our laboratory to evaluate the pharmacological activities of the target compounds of the lead plants.

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REFERENCES

(1) Apel, K.; Hirt, H. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. *Annu. Rev. Plant Biol.* **2004**, *55*, 373–399.

(2) Tang, S. Y.; Whiteman, M.; Peng, Z. F.; Jenner, A.; Yong, E. L.; Halliwell, B. Characterization of antioxidant and antiglycation properties and isolation of active ingredients from traditional Chinese medicines. *Free Radical Biol. Med.* **2004**, *36*, 1575–1587.

(3) Cai, Y.; Luo, Q.; Sun, M.; Corke, H. Antioxidant activity and phenolic compounds of 112 traditional Chinese medicinal plants associated with anticancer. *Life Sci.* **2004**, *74*, 2157–2184.

(4) Rice-Evans, C. A.; Miller, N. J.; Paganga, G. Structure-antioxidant activity relationships of flavonoids and phenolic acids. *Free Radical Biol. Med.* **1996**, *20* (7), 933–956.

(5) Yoon, J. H.; Baek, S. J. Molecular targets of dietary polyphenols with anti-inflammatory properties. *Yonsei Med. J.* **2005**, *46* (5), 585.

(6) Feldmann, M.; Maini, S. Role of cytokines in rheumatoid arthritis: an education in pathophysiology and therapeutics. *Immunol. Rev.* **2008**, *223*, 7–19.

(7) Lee, Y. S.; Han, O. K.; Park, C. W.; Yang, C. H.; Jeon, T. W.; Yoo, W. K.; Kim, S. H.; Kim, H. J. Pro-inflammatory cytokine gene expression and nitric oxide regulation of aqueous extracted *Astragali radix* in RAW 264.7 macrophage cells. *J. Ethnopharmacol.* **2005**, *100*, 289–294.

(8) Wang, C.; Schuller Levis, G. B.; Lee, E. B.; Levis, W. R.; Lee, D. W.; Kim, B. S.; Park, S. Y.; Park, E. Platycodin D and D3 isolated from the root of *Platycodon grandiflorum* modulate the production of nitric oxide and secretion of TNF-α in activated RAW 264.7 cells. *Int. Immunopharm.* **2004**, *4*, 1039–1049.

(9) Moon, J. K.; Shibamoto, T. Antioxidant assays for plant and food components. *J. Agric. Food Chem.* **2009**, *57* (5), 1655–1666.

(10) Azevedo, F.; Marques, F.; Fokt, H.; Oliveira, R.; Johansson, B. Measuring oxidative DNA damage and DNA repair using the yeast comet assay. *Yeast* **2011**, *28*, 55−61.

(11) Lee, J.; Godon, C.; Lagniel, G.; Spector, D.; Garin, J.; Labarre, J.; Toledano, M. B. Yap1 and Skn7 control two specialized oxidative stress response regulons in yeast. *J. Biol. Chem.* **1999**, *274* (23), 16040.

(12) Cordell, G. A.; Beecher, C. W.; Pezzuto, J. M. Can ethnopharmacology contribute to the development of new anticancer drugs. *J. Ethnopharmacol.* **1991**, *32*, 117–133.

(13) Cox, P. A. The ethnobotanical approach to drug discovery: strengths and limitations. *Ciba Found. Symp.* **1994**, *185*, 25–41.

(14) Cho, W. C. S. *Supportive Cancer Care with Chinese Medicine*; Springer Verlag: Berlin, Germany, 2010.

(15) Huang, Y. C.; Guh, J. H.; Teng, C. M. Denbinobin-mediated anticancer effect in human K562 leukemia cells: role in tubulin polymerization and Bcr-Abl activity. *J. Biomed Sci.* **2005**, *12* (1), 113–121.

(16) Cicco, N.; Lanorte, M. T.; Paraggio, M.; Viggiano, M.; Lattanzio, V. A reproducible, rapid and inexpensive Folin-Ciocalteu micromethod in determining phenolics of plant methanol extracts. *Microchem. J.* **2009**, *91*, 107–110.

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

(18) Blois, M. S. Antioxidant determinations by the use of a stable free radical. *Nature* **1958**, *181*, 1199–1200.

(19) Wu, M. J.; O'Doherty, P. J.; Fernandez, H. R.; Lyons, V.; Rogers, P. J.; Dawes, I. W.; Higgins, V. J. An antioxidant screening assay based on oxidant induced growth arrest in *Saccharomyces cerevisiae*. *FEMS Yeast Res.* **2011**, *11*, 379–387.

(20) Shanmugam, K.; Holmquist, L.; Steele, M.; Stuchbury, G.; Berbaum, K.; Schulz, O.; Benavente-García, O.; Castillo, J.; Burnell, J.; Garcia Rivas, V.; Dobson, G.; Münch, G. Plant-derived polyphenols attenuate lipopolysaccharide-induced nitric oxide and tumour necrosis factor production in murine microglia and macropgages. *Mol. Nutr. Food Res.* **2008**, *52*, 427–438.

(21) Kim, D. O.; Jeong, S. W.; Lee, C. Y. Antioxidant capacity of phenolic phytochemicals from various cultivars of plums. *Food Chem.* **2003**, *81* (3), 321–326.

(22) Cai, Y.; Sun, M.; Xing, J.; Corke, H. Antioxidant phenolic constituents in roots of *Rheum officinale* and *Rubia cordifolia*: structure-radical scavenging activity relationships. *J. Agric. Food Chem.* **2004**, *52*, 7884–7890.

(23) Kim, J. M.; Yun-Choi, H. S. Anti-platelet effects of flavonoids and flavonoid-glycosides from *Sophora japonica*. *Arch. Pharm. Res.* **2008**, *31* (7), 886–890.

(24) Hsu, C. Y.; Chan, Y. P.; Chang, J. Antioxidant activity of extract from *Polygonum cuspidatum*. *Biol. Res.* **2007**, *40* (1), 13–21.

(25) da Silva Oliveira, C.; Maciel, L. F.; Miranda, M. S.; da Silva Bispo, E. Phenolic compounds, flavonoids and antioxidant activity in different cocoa samples from organic and conventional cultivation. *Br. Food J.* **2011**, *113* (9), 1094–1102.

(26) Huang, W. H.; Lee, A. R.; Yang, C. H. Antioxidative and anti-inflammatory activities of polyhydroxyflavonoids of *Scutellaria baicalensis* Georgi. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 2371–2380.

(27) Feng, S. X.; Xu, L. X.; Wu, X.; Hao, J.; Qiu, S. X.; Wei, X. Y. A new coumarin from *Sarcandra glabra*. *Fitoterapia* **2010**, *81*, 472–474.

(28) Ng, T.; Liu, F.; Wang, H. The antioxidant effects of aqueous and organic extracts of *Panax quinquefolium*, *Panax notoginseng*, *Codonopsis pilosula*, *Pseudostellaria heterophylla* and *Glehnia littoralis*. *J. Ethnopharmacol.* **2004**, *93*, 285–288.

(29) Li, Y.; Wo, J. M.; Liu, Q.; Li, X.; Martin, R. C. G. Chemoprotective effects of *Curcuma aromatica* on esophageal carcinogenesis. *Ann. Surg. Oncol.* **2009**, *16*, 515–523.

(30) Huang, W. H.; Lee, A. R.; Yang, C. H. Antioxidative and anti-inflammatory activities of polyhydroxyflavonoids of *Scutellaria baicalensis* Georgi. *Biosci., Biotechnol., Biochem.* **2006**, *70*, 2371–2380.

(31) Wang, Y.; Zhang, S. Y.; Ma, X. F.; Tian, W. X. Potent inhibition of fatty acid synthase by *Parasitic loranthus* [*Taxillus chinensis* (DC.) Danser] and its constituent avicularin. *J. Enzym. Inhib. Med. Chem.* **2006**, *21*, 87–93.

(32) Yen, G. C.; Hsieh, C. L. Antioxidant activity of extracts from Du-zhong (*Eucommia ulmoides*) toward various lipid peroxidation models in vitro. *J. Agric. Food Chem.* **1998**, *46*, 3952–3957.

(33) Li, C. Q.; He, L. C.; Dong, H. Y.; Jin, J. Q. Screening for the anti-inflammatory activity of fractions and compounds from *Atractylodes macrocephala* koidz. *J. Ethnopharmacol.* **2007**, *114*, 212–217.

(34) Kim, B. H.; Chung, E. Y.; Ryu, J. C.; Jung, S. H.; Min, K. R.; Kim, Y. Anti-inflammatory mode of isoflavone glycoside sophoricoside by inhibition of lnterleukin-6 and cyclooxygenase-2 in inflammatory response. *Arch. Pharm. Res.* **2003**, *26*, 306–311.

(35) Hsu, C. Y.; Chan, Y. P.; Chang, J. Antioxidant activity of extract from *Polygonum cuspidatum*. *Biol. Res.* **2007**, *40*, 13–21.

(36) Okuyama, E.; Hasegawa, T.; Matsushita, T.; Fujimoto, H.; Ishibashi, M.; Yamazaki, M. Analgesic components of saposhnikovia root (*Saposhnikovia divaricata*). *Chem. Pharm. Bull.* **2001**, *49*, 154–160.

(37) Li, A. R.; Zhu, Y.; Li, X. N.; Tian, X. J. Antimicrobial activity of four species of *Berberidaceae*. *Fitoterapia* **2007**, *78*, 379–381.

(38) Sohn, H. Y.; Son, K.; Kwon, C. S.; Kwon, G. S.; Kang, S. Antimicrobial and cytotoxic activity of 18 prenylated flavonoids isolated from medicinal plants: *Morus alba* L., *Morus mongolica* Schneider, *Broussnetia papyrifera* (L.) Vent, *Sophora flavescens* Ait and *Echinosophora koreensis* Nakai. *Phytomedicine* **2004**, *11*, 666–672.

(39) Chen, J.; Cui, G.; Liu, J.; Tan, R. Pinelloside, an antimicrobial cerebroside from *Pinellia ternata*. *Phytochemistry* **2003**, *64*, 903–906.