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Antioxidant Capacity of 55 Medicinal Herbs Traditionally Used to Treat the Urinary System: A Comparison Using a Sequential Three-Solvent Extraction Process

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ABSTRACT

Background: The prevalence of chronic renal disease exceeds 10% in industrialized societies. Oxidative damage is thought to be one of the main mechanisms involved in nearly all chronic renal pathologies.

Objective: We aimed to use the oxygen radical absorbance capacity (ORAC) method and a sequential multi-solvent extraction process to compare the *in vitro* antioxidant capacity of 55 medicinal herbs and prioritize them for *in vivo* studies investigating the value of herbal therapies in the treatment of renal disorders.

Methods: The herbs were chosen on the basis of their traditional use in kidney or urinary system disorders, or because they have attracted the attention of recent investigations into renal pathologies. The three solvents used for extraction were ethyl acetate, methanol, and 50% aqueous methanol. *Silybum marianum* (milk thistle) seed and *Camellia sinensis* (tea) leaf, both known to possess high antioxidant capacity, were included for comparison.

Results: Twelve of the 55 herbs were comparable to or exceeded ORAC levels of milk thistle seed or tea leaf. The highest radical-scavenging activity was found in *Olea europaea* (olive leaf), *Cimicifuga racemosa* (black cohosh), *Rheum palmatum* (rhubarb), *Glycyrrhiza glabra* (licorice), and *Scutellaria lateriflora* (Virginia skullcap).

Conclusions: The antioxidant capacity of many of the herbs studied may, at least in part, be responsible for their reputation as being protective of organs of the urinary system. Overall, the combined ORAC values for the methanol and aqueous methanol extracts comprised 84% of the total ORAC value. Sequential extraction with solvents of different polarities may be necessary to fully extract the antioxidant principles from medicinal plants.

INTRODUCTION

The prevalence of chronic renal disease (CRD) exceeds 10% in industrialized societies.[1] Oxidative damage is thought to be a major mechanism involved in the development of numerous renal disorders including diabetic nephropathy,[2] ischemic nephropathy,[3] some types of iatrogenic nephropathy,[4,5] Balkan endemic nephropathy,[4] IgA nephropathy,[6] and others.[7] As the renal condition worsens and the patient enters chronic renal failure, the resulting uremia leads to systemic oxidative stress and damage to other organs.[8] Presently, it is not known whether antioxidant therapy might be useful in preventing or delaying the progression of these diseases. In experimental nephritis, antioxidants have been found to be beneficial in some[5,9,10] but not all studies. Likewise, in humans, results of antioxidant supplementation in the treatment of renal disease have varied.[7,11]

Plants contain a diverse range of bioactive molecules, many of which have antioxidant properties. However, be-

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cause of varying polarity of these constituents, they may not always be exhaustively extracted by a single solvent. Only a few antioxidant studies have aimed to extract herbs in a sequential manner using solvents of different polarity.[12–15] These studies have been beneficial in identifying antioxidant fractions and providing information regarding the most appropriate combination of solvents for the extraction of the antioxidant constituents from the plants studied. To our knowledge, there have been no antioxidant studies aimed at investigating a wide range of medicinal plants using a sequential extraction by three solvents of different polarity.

The primary aim of this study was to compare the *in vitro* radical-scavenging capacity of 55 herbs selected on the basis of their traditional use in kidney or urinary system disorders or on the recent interest they have attracted from investigators of renal pathologies. This comparison will help prioritize herbs for future *in vivo* studies investigating the efficacy of herbal therapies in the treatment of renal pathologies. The secondary aim was to employ a sequential multisolvent extraction process with a view to optimizing the extraction of antioxidant compounds from plants and comparing the activity of different solvent extracts. Such a comprehensive multistep extraction provides a more complete picture of the plants' total antioxidant activity, helping to ensure that potential candidates for further study are not overlooked, while the information on the polarity of the active principles from each plant will help streamline further studies. We therefore extracted each herb sequentially in three solvents, in order of increasing polarity. The antioxidant capacity of each extract as well as the total antioxidant capacity of each herb is presented here.

MATERIALS AND METHODS

Chemicals

Sodium dihydrogen phosphate (NaH_2PO_4); disodium hydrogen phosphate (Na_2HPO_4); butylated hydroxytoluene ($C_{15}H_{24}O$); and fluorescein sodium salt were purchased from Sigma Aldrich Chemical Co. (Castle Hill, Australia). 2,2′-Azobis(2-amidinopropane) dihydrochloride (AAPH) was purchased from Wako Chemicals USA Inc. (Richmond, Virginia). 6-Hydroxy-2,5,7,8-tetramethylchroman-2-carboxylic acid (Trolox) was obtained from Fluka Chemie GmbH (Buchs, Switzerland). Ethyl acetate, methanol, and acetone were of high-performance liquid chromatography grade, purchased from LabScan, Analytical Services (Brisbane, Australia). All water used was of Milli-Q quality (Millipore Corp., Bedford, Massachusetts).

Plant material and extraction

Crude herbal materials listed in Table 1 were obtained from the Medicinal Plant Garden at Southern Cross University and from other sources and were authenticated to pharmacopoeial monographs or other scientific literature by a pharmacognosist (H. Wohlmuth). In some instances, the botanical drugs were obtained from reliable suppliers but not authenticated independently (Table 1). Voucher samples of all herbs were assigned a reference number and deposited in the Medicinal Plant Herbarium at Southern Cross University (Table 1). The dried plant material was ground to a powder using a Retsch SM2000 (Retsch GmbH, Haan, Germany) mill fitted with a 0.5-mm screen and extracted by a three-solvent sequential process. Ground material (2 g) was sonicated (10 minutes) in ethyl acetate (20 mL) and filtered (Whatman No. 3, gravity filtration; Whatman plc, Brentford, Middlesex, UK). After a second and third extraction with that solvent, the filtrate was dried in a rotary vacuum centrifuge (Christ B-RVC, Harz, Germany), redissolved in acetone and stored (−17°C). The process was repeated with the residue sonicated in 100% methanol, and finally 50% aqueous methanol (v:v) in the same manner, except that the resulting filtrates were redissolved (20 mg/mL) in the same type of solvent used in the extraction.

ORAC assay

Antioxidant capacity was determined using the oxygen radical absorbance capacity ($ORAC_{FL}$) assay. This assay quantified the antioxidant activity of the herb extracts on fluorescein in the presence of free radicals generated by AAPH. Ethyl acetate extracts were assayed in the lipophilic ORAC assay, and methanol and aqueous methanol extracts were assayed in the hydrophilic ORAC assay.[16] The assay was carried out in 96-well polypropylene fluorescence plates (Greiner bio-one, Frickenhausen, Germany) with a final volume of 200 μL. The concentration of solvent in the samples was always matched in the blank and standard. Assays were conducted at pH 7.0 with Trolox (6.25, 12.5, 25, and 50 μmol/L for lipophilic assays; 12.5, 25, 50 and 100 μmol/L hydrophilic assays) as the standard and 75 mmol/L phosphate buffer as the blank. After the addition of AAPH, the plate was placed immediately in a Wallac Victor 2 1420 multilabel counter (Perkin-Elmer, Turku, Finland) preheated to 37°C. The plate was shaken in an orbital manner for 10 seconds and the fluorescence was read at 1-minute intervals for 35 minutes at the excitation wavelength of 485 nm and emission wavelength of 520 nm. Area-under-the-curve was calculated for each sample using Wallac Workout 1.5 software (Perkin-Elmer, Turku, Finland). Final computation of results was made by taking the difference of areas-under-the-decay curves between blank and sample and/or standard (Trolox) and expressing this in micromoles of Trolox equivalents (TE) per gram dry weight (dw) of crude starting material (μmol TE/g dw).

RESULTS

ORAC values for the three extracts of each herb plus the total values are listed in Table 2. The herbal samples demon-

Table 1. General Information Regarding Plants Studied

Latin name	*Common name*	*Part(s)*	*Voucher number*
Achillea millefolium spp. pannonica	Yarrow	Aerial	NCM-03-042
Agathosma betulina[a]	Buchu	Leaf	CP-04-0135
Agrimoni eupatoria	Agrimony	Aerial	NCM-04-076
Andrographis paniculata	Andrographis	Aerial	NCM-04-056
Angelica archangelica	Angelica	Root	NCM-04-033
Angelica sinensis[a]	Danggui	Root	CP-04-0079
Apium graveolens	Celery	Seed	NCM-D-04-062
Arctostaphylos uva-ursi[b]	Bearberry	Leaf	NCM-D-05-024
Artemisia arborescens	Tree wormwood	Leaf	NCM-04-017
Astragalus membranaceus[b]	Astragalus	Root	CP-04-206
Burpleurum falcatum[a]	Bupleurum	Root	NCM-06-016
Camellia sinensis	Tea	Leaf	NCM-04-055
Centella asiatica	Gotu kola	Aerial	NCM-04-009
Cimicifuga racemosa	Black cohosh	Root/rhizome	NCM-04-106
Cinnamomum sp.	Cinnamon	Bark	NCM-04-029
Cordyceps sinensis[c]	Cordyceps	Fungus and caterpillar host	CP-04-0215
Curcuma longa	Turmeric	Rhizome	NCM-D-05-025
Dioscorea villosa[a]	Wild yam	Root	CP-04-0059
Eleutherococcus gracilistylus		Root	NCM-04-054
Elymus repens[a]	Couch grass	Rhizome	CP-04-0108
Fagopyrum esculentum[c]	Buckwheat	Fruit	NCM-D-05-028
Ganoderma lucidum[c]	Reishi mushroom	Basidiocarp	CP-04-218
Glycyrrhiza glabra	Licorice	Root	NCM-04-029
Inula helenium	Elecampane	Root	NCM-04-026
Iris versicolor	Blue flag	Rhizome	NCM-04-153
Juniperus communis	Juniper	Frit	NCM-D-04-026
Levisticum officinale[b]	Lovage	Root	NCM-D-05-027
Nasturtium officinale	Watercress	Aerial	NCM-03-035
Ocimum basilicum	Basil	Leaf	NCM-04-114
Olea europaea	Olive	Leaf	NCM-04-063
Panax ginseng	Korean ginseng	Root	NCM-D-04-018
Papaver somniferum	Opium poppy	Seed	NCM-D-05-026
Perilla frutescens	Perilla	Aerial	NCM-04-025
Petroselinum crispum	Parsley	Leaf	NCM-03-033
Pulsatilla spp.	Pasque flower	Aerial	NCM-04-152
Rheum palmatum	Chinese rhubarb	Root/rhizone	NCM-D-04-114
Ruscus aculeatus[b]	Butcher's broom	Rhizome	CP-04-0195
Salvia miltiorrhiza[c]	Danshen	Root	CP-04-0216
Salvia officinalis	Sage	Leaf	NCM-D-04-028
Scutellaria lateriflora	Virginia skullcap	Aerial	NCM-02-001
Serenoa repens	Saw palmetto	Fruit	NCM-D-04-025
Silybum marianum	Milk thistle	Seed	CP-04-0061
Solidago canadensis	Canada golden rod	Aerial	NCM-04-021
Solidago virgaurea	Golden rod	Aerial	NCM-04-101
Spirunlina platensis[a]	Spirulina	Organism	NCM-D-05-023
Stachys officinalis	Wood betony	Aerial	NCM-04-133
Taraxacum officinale	Dandelion	Leaf	NCM-04-028
Taraxacum officinale	Dandelion	Root	NCM-04-28
Turnera diffusa[a]	Damiana	Aerial	CP-04-080
Uncaria tomentosa[a]	Cats claw	Root bark	CP-04-081
Urtica dioica	Stinging nettle	Aerial	NCM-04-052
Verbascum thapsus	Woolly mullein	Aerial	NCM-05-002
Viscum album[a]	Mistletoe	Aerial	CP-04-0124
Withania somnifera	Withania	Root	NCM-04-136
Zea mays[a]	Corn silk	Style/stigma	CP-04-109

Unless otherwise indicated, all herbs were authenticated to pharmacopoeial monographs or other scientific literature by a pharmacognosist (H. Wohlmuth).

[a]Obtained from Austral Herbs Pty Ltd, Australia but not authenticated independently.

[b]Obtained from MediHerb Pty Ltd, Warwick, Qld, Australia but not authenticated independently.

[c]Obtained from Wing Hing Chinese Herbs, Fortitude Valley, Qld, Australia but not authenticated independently.

Table 2. Oxygen Radical Absorbance Capacity (ORAC) Values of Herbs by Sequential Solvent Extraction

	ORAC[a] *Ethyl acetate extract*	*ORAC*[a] *Methanol extract*	*ORAC*[a] *Aqueous methanol (1:1; v:v) extract*[c]	*Total ORAC*[a] *(3 sequential extractions combined)*
Achillea millefolium spp. pannonica	22.72 ± 2.68	61.60 ± 4.13	174.92 ± 15.50	259.25
Agathosma betulina[a]	182.54 ± 13.58	127.02 ± 17.25	78.63 ± 6.06	388.19
Agrimoni eupatoria	9.19 ± 0.57	97.72 ± 7.77	170.90 ± 6.82	277.81
Andrographis paniculata	5.25 ± 0.40	168.10 ± 11.88	346.61 ± 21.89	519.97
Angelica archangelica	259.51 ± 10.54	42.44 ± 2.89	46.34 ± 3.96	348.29
Angelica sinensis[a]	47.50 ± 2.57	74.61 ± 4.69	122.57 ± 11.67	244.68
Apium graveolens	60.63 ± 5.66	72.92 ± 4.66	61.55 ± 6.16	195.11
Arctostaphylos uva-ursi[b]	N/A	184.36 ± 10.61	517.95 ± 29.15	702.32
Artemisia arborescens	43.68 ± 2.32	237.20 ± 6.81	261.17 ± 17.21	542.05
Astragalus membranaceus[b]	27.47 ± 1.00	16.04 ± 1.15	134.21 ± 13.17	177.73
Burpleurum falcatum[a]	5.11 ± 0.41	47.40 ± 3.31	69.63 ± 7.10	122.15
Camellia sinensis	3.96 ± 0.42	577.49 ± 46.36	45.68 ± 6.22	627.14
Centella asiatica	N/A	496.84 ± 50.10	202.94 ± 21.76	699.78
Cimicifuga racemosa	N/A	42.59 ± 4.37	1222.36 ± 153.24	1264.95
Cinnamomum spp.	85.39 ± 6.38	123.48 ± 14.28	71.67 ± 9.59	280.54
Cordyceps sinensis[c]	N/A	83.49 ± 9.87	33.79 ± 4.63	117.28
Curcuma longa	466.81 ± 42.46	61.13 ± 7.49	29.45 ± 1.14	557.40
Dioscorea villosa[a]	14.48 ± 0.73	171.57 ± 14.68	196.08 ± 16.77	382.14
Eleutherococcus gracilistylus	N/A	51.96 ± 6.22	31.85 ± 2.76	83.81
Elymus repens[a]	8.37 ± 1.00	119.43 ± 10.42	11.09 ± 0.77	138.88
Ganoderma lucidum[c]	7.46 ± 0.98	30.29 ± 1.29	54.68 ± 7.91	92.44
Glycyrrhiza glabra	196.44 ± 7.48	416.93 ± 41.72	416.08 ± 41.99	1029.45
Inula helenium	12.42 ± 0.95	22.70 ± 1.50	25.17 ± 1.77	60.30
Iris versicolor	11.53 ± 0.97	112.10 ± 13.02	145.02 ± 8.03	268.66
Juniperus communis	37.79 ± 2.11	55.55 ± 7.95	13.27 ± 1.44	106.61
Levisticum officinale[b]	7.69 ± 1.11	50.17 ± 6.39	21.06 ± 2.81	78.93
Nasturtium officinale	N/A	214.43 ± 9.46	457.98 ± 61.98	672.40
Ocimum basilicum	38.44 ± 4.20	171.82 16.54	314.50 ± 37.64	524.76
Olea europaea	158.20 ± 13.70	860.85 ± 66.36	261.53 ± 17.01	1280.59
Panax ginseng[a]	1.22 ± 0.141	20.79 ± 1.10	16.17 ± 1.51	38.18
Papaver somniferum	3.35 ± 0.41	10.72 ± 0.92	2.05 ± 0.13	16.13
Perilla frutescens	1.130 ± 0.12	152.23 ± 9.75	86.48 ± 5.49	239.84
Petroselinum crispum	8.83 ± 1.22	291.11 ± 35.37	445.00 ± 51.83	744.95
Fagopyrum esculentum[c]	0.22 ± 0.02	13.70 ± 0.92	2.71 ± 0.23	16.64
Pulsatilla spp.	7.20 ± 0.32	50.62 ± 5.93	287.40 ± 21.71	345.22
Rheum palmatum	679.63 ± 24.99	336.98 ± 41.14	178.68 ± 8.46	1195.30
Ruscus aculeatus[b]	79.53 ± 6.40	183.65 ± 9.07	138.53 ± 12.34	401.72
Salvia miltiorrhiza[c]	6.98 ± 0.75	13.89 ± 1.61	118.35 ± 10.59	139.23
Salvia officinalis	12.17 ± 1.14	120.12 ± 11.87	221.75 ± 18.54	354.04
Scutellaria lateriflora	269.99 ± 24.78	123.17 ± 3.69	634.09 ± 42.73	1027.26
Serenoa repens	0.91 ± 0.06	48.50 ± 2.07	69.95 ± 3.7	119.36
Silybum marianum	516.86 ± 31.44	26.34 ± 2.21	10.70 ± 0.79	553.91
Solidago canadensis	1.55 ± 0.15	145.63 ± 12.07	209.18 ± 12.27	356.36
Solidago virgaurea	0.48 ± 0.02	107.26 ± 14.22	3.02 ± 0.06	110.75
Spirunlina platensis[a]	1.07 ± 0.09	37.51 ± 2.18	7.03 ± 0.19	45.62
Stachys officinalis	2.68 ± 0.04	110.78 ± 6.39	148.84 ± 6.65	262.30
Taraxacum officinale	13.33 ± 1.16	40.50 ± 4.96	42.83 ± 4.25	96.66
Taraxacum officinale	48.65 ± 4.56	49.15 ± 1.72	54.20 ± 2.08	152.00
Turnera diffusa[a]	47.32 ± 4.75	783.58 ± 113.08	92.84 ± 9.35	923.75
Uncaria tomentosa[a]	23.24 ± 2.77	139.36 ± 8.15	60.92 ± 4.50	223.53
Urtica dioica	N/A	71.12 ± 6.33	359.30 ± 44.06	430.42
Verbascum thapsus	117.55 ± 17.40	300.65 ± 35.00	189.20 ± 15.62	607.41
Viscum album[a]	35.43 ± 3.43	219.88 ± 17.77	50.06 ± 4.44	305.37
Withania somnifera	1.48 ± 0.18	47.58 ± 5.12	35.80 ± 3.45	84.87
Zea mays[a]	N/A	17.84 ± 2.50	41.90 ± 6.02	59.74

[a]All ORAC values in μmol Trolox equivalent per g of dried starting material (μmol TE/g dw).
[b]Methanol extract of the plant residue after extraction with ethyl acetate.
[c]Aqueous methanol extract of the plant residue after extraction with ethyl acetate followed by methanol.
SD, standard deviation ($n = 6$); N/A, not available because of insufficient yield to accurately determine antioxidant activity.

strated a wide range of antioxidant activity: from 16.13 to 2487.37 μmol TE/g dw when the ORAC values for all three extracts were totaled. Twelve of the samples demonstrated higher antioxidant capacity than either *Silybum marianum* seed or *Camellia sinensis* leaf. The highest activity was found in *Olea europaea* leaf, *Cimicifuga racemosa* root/rhizome, *Rheum palmatum* root/rhizome, and *Glycyrrhiza glabra* root and *Scutellaria lateriflora* aerial parts. In eight instances, there was insufficient material to accurately determine the antioxidant activity of the ethyl acetate extract from the 2 g of herb extracted (Table 2).

Overall, 15.7%, 39.1%, and 45.2% of the total antioxidant activity measured were attributable to the ethyl acetate, methanol, and aqueous methanol extracts, respectively. Therefore, 84.3% of the total antioxidant capacity measured from these medicinal herbs was extracted with the most polar solvents, methanol and aqueous methanol.

DISCUSSION

Given the increased prevalence of CRD combined with the role that oxidation plays in the development and progression of chronic renal pathologies, we investigated 55 herbs that have been used traditionally to treat the kidneys and urinary tract. Ten of the samples demonstrated higher antioxidant capacity than both *S. marianum* seed and *C. sinensis* leaf, both of which are known to have potent antioxidant capacity.[17,18] *O. europaea* leaf, found by others to contain a mixture of phenolic compounds with significant antioxidant activity,[19] demonstrated the highest ORAC values at 1280.58 μmol TE/g dw. *C. racemosa*, an herb used for renal and urinary tract disorders by Native Americans,[20] had a total ORAC value of 1264.95 μmol TE/g dw. Others have found this herb to contain very potent antioxidant compounds, six of which reduced menadione-induced DNA damage in cultured breast cells.[21] The main antioxidant compounds found in that study were methyl caffeate, ferulic acid, and caffeic acid. The extraction yield from this root is also high (167 mg/g after a two-solvent extraction[21] and 267 mg/g in the present study), which obviously contributes significantly to results presented in relation to a given amount of starting material. Using this three-solvent process, *R. palmatum* root/rhizome and *G. glabra* root, both of which are used in the treatment of renal disorders in Chinese, Japanese, and Western Traditional Medicine,[22] had total ORAC values of 1195 μmol TE/g dw and 1029 μmol TE/g dw, respectively. Previous investigations have reported *Rheum officinale* (another species considered to be medicinally interchangeable with *R. palmatum*) to contain numerous phenolic constituents with high antioxidant activity,[23] and *G. glabra* to have antioxidant activity comparable to that of ginkgo,[24] an herb found in several studies to have strong antioxidant activity (reviewed in ref. 25) The aerial parts of *S. lateriflora*, an herb that is included in the Japanese polyherbal formulae for CRD (Saire-to and Sho-saiko-to)[22] had a total ORAC value of 1027 μmol TE/g dw. In comparison, our sample of *C. sinensis* leaf had a value of 627.13 μmol TE/g dw while others found that different samples of *C. sinensis* leaf ranged from 235 to 1526 μmol TE/g dw.[17]

An unexpected finding was the relatively low antioxidant capacity of some extracts. One example is *Astragalus membranaceus* (177.72 μmol TE/g dw), used by Traditional Chinese practitioners for renal disorders. This herb has demonstrated high antioxidant activity in protecting against intestinal mucosal reperfusion injury in rats.[26] Because this was an *in vivo* study, it is possible that antioxidant activity was modified within the whole animal. Although we have analyzed the lipophilic and hydrophilic radical-quenching capacity using the ORAC assay, which has been found to be the most relevant for biologic samples,[27] limitations of *in vitro* antioxidant assays include the fact that they do not account for bioavailability, retention of antioxidants by tissues, and reactivity *in vivo*.[28]

We chose a multisolvent extraction technique because it is the preferred method when further work on the plant is expected.[29] This technique provides information regarding the most appropriate combination of solvents for the extraction of the antioxidant constituents from herbs studied. Other advantages include the simplification of the biomass and the fact that there is little potential degradation of the constituents because of the ambient temperature of the solvents.[29] Sonication is a technique that has been used for extraction of desired constituents of plants for many years.[30] The method disrupts the cell membranes and increases solubility while not altering the molecular structure of the constituents.[31,32] Few investigations have used a similar multisolvent sequential extraction technique in studies of plant antioxidant activity. In each of the four studies that have used this technique, it was found that the extracts from the most polar solvent (heated water) and least polar solvents (hexane, *t*-butyl methyl ether, or petroleum ether) generally had the lowest antioxidant activity.[12–15] After the aqueous methanol extraction, it was found that the remaining residue generally contains high-molecular-weight carbohydrates that add bulk to the extract but have little antioxidant activity when assayed *in vitro*.[26] The very hydrophilic solvents are generally less effective at extracting phenolic compounds, and levels of these compounds usually correlate well with the antioxidant activity of the herb or extract.[33] Because of these observations and our interest in antioxidant activity, we chose three solvents that were not at the extremes of polarity.

Because this three-solvent sequential extraction technique for studying antioxidant activity of herbs has not been used to assess the ORAC value of herbs before, it is not surprising that our results differed from that obtained by other researchers using ORAC. Biologically active plant metabolites can vary significantly within a species, both quantita-

tively and qualitatively. Such variation can be the product of genetic, environmental, ontogenic, or biologic factors. Conditions of drying and storage can also significantly affect the biologic activity of dried plant samples. The use of different extraction solvents can clearly also be the cause of seemingly disparate values in a bioassay.

Our result for *Ocimum basilicum* leaf (524 μmol TE/g dw) was more than 10-fold higher than one previously reported (48 μmol TE/g dw) using 80:20 v/v acetone/perchloric acid (5%) as the extraction solvent.[34] We found that 59.99% of the antioxidant capacity of basil was extracted with the most polar solvent (aqueous methanol). This example highlights the fact that significant antioxidant activity may not be extracted if the most polar solvent used in the extraction process is anhydrous methanol. Another study found the ORAC value for *Curcuma longa* rhizome to be in the range of 10.86–25.16 μmol TE/g fresh weight,[11] whereas we found the total ORAC value from three sequential extractions to be 557.4 μmol TE/g dw. Our laboratories have found that *C. longa* rhizome contains an average of 77% moisture (unpublished data). Correcting for fresh weight, the dry weight equivalent of the published samples would range from 45 to 103 μmol TE/g dw. It is possible that some of the antioxidant capacity was missed by these workers as they extracted the rhizome in water or ethanol. In the present analysis, 91.97% of the antioxidant capacity was found in the ethyl acetate fraction, emphasizing the need for at least one polar and one nonpolar solvent being included when doing antioxidant screening tests of herbs or foods.

In conclusion, 55 herbs of potential interest in the context of renal or urinary tract pathologies were tested for ORAC using polar and nonpolar solvents. Twelve herbs are primary candidates for further *in vivo* research focusing on the efficacy of herbal therapies in renal diseases involving oxidative damage. The results from the three-solvent sequential extraction technique for screening herbs suggest that medicinal herbs should be extracted by at least two solvents of varying polarity for optimum extraction, thereby providing a more complete assessment of the total antioxidant activity.

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