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Original Article

Antioxidant activity of 45 Chinese herbs and the relationship with their TCM characteristics

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Here, 45 Chinese herbs that regulate blood circulation were analyzed for antioxidant activity using the oxygen radical absorbance capacity (ORAC) assay. A recent publication by Ou *et al*. identified a close relationship between *in vitro* antioxidant activity and classification of Chinese herbs as *yin* or *yang*. The 45 Chinese herbs in this study could be assigned the traditional characteristics of natures (cold, cool, hot and warm), flavors (pungent, sweet, sour, bitter and salty) and functions (arresting bleeding, promoting blood flow to relieve stasis, nourishing blood and clearing away heat from blood). These characteristics are generalized according to the theory of *yin* and *yang*. We identified a broad range, 40–1990 µmol Trolox Equivalent/g herbs, of antioxidant activity in water extracts. There was no significant correlation between ORAC values and natures or functions of the herbs. There was a significant relationship between flavors and ORAC values. Bitter and/or sour herbs had the highest ORAC values, pungent and/or sweet herbs the lowest. Other flavors had intermediate values. Flavors also correspond with the *yin*/*yang* relationship and our results are supportive of the earlier publication. We reported for the first time antioxidant properties of many Chinese herbs. High antioxidant herbs were identified as *Spatholobus suberectus* vine (1990 µmol TE/g), *Sanguisorba officinalis* root (1940 µmol TE/g), *Agrimonia pilosa* herb (1440 µmol TE/g), *Artemisia anomala* herb (1400 µmol TE/g), *Salvia miltiorrhiza* root (1320 µmol TE/g) and *Nelembo nucifera* leaf (1300 µmol TE/g). Antioxidant capacity appears to correlate with the flavors of herbs identified within the formal TCM classification system and may be a useful guide in describing their utility and biochemical mechanism of action.

Keywords: flavor – function – herbs – nature – oxygen radical absorbance capacity (orac) – traditional Chinese medicine

Introduction

Traditional Chinese Medicine (TCM) has relied on experiential evidence and traditional healing manuscripts. Prior to the wider use of TCM theory and medicines in other medical systems, particularly western biomedical medicine, research is required to understand the biochemical basis for TCM classification systems for drugs and the mechanism of action of these drugs. A recent publication by Ou *et al*. (1) identified a close relationship between *in vitro* antioxidant activity classification of Chinese herbs as *yin* and *yang*.

In TCM's long course of development, it has absorbed the quintessence of classical Chinese philosophy, culture and science and summarized the experience of the Chinese people in fighting diseases (2). This experiential evidence as a basis of TCM has resulted in a totally different medical theory compared with western conventional medicine (3). TCM believes that the different characters of herbs are employed to treat diseases, rectify

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the hyperactivity or hypoactivity of *yin* or *yang* and help the body restore its normal physiologic functions. All herbs possess four natures and five flavors. The four natures—cold, hot, warm and cool are summarized mainly from the body's response after Chinese herbs are taken, which are so defined in relation to the properties, cold or heat of the diseases treated. In addition, there are also some herbs known as neutral ones, whose cold or hot nature is not so remarkable and whose action is relatively mild, but these herbs still have differences in their tendencies to cool or warm so that they are still in the range of four natures. The five flavors are the pungent, sweet, sour, bitter and salty that can be tasted with the tongue. With the development of the theory dealing with the medicinal properties, the flavors could best be described as abstract concepts, as the flavor definitions have arisen more from observations of the clinical actions of the herbs than from the taste sensations.

Most herbs contain a combination of a number of flavors, for example 'bitter with sweet and pungent' (4). The nature and the flavor are two kinds of medicinal properties that every Chinese herb possesses. Modern scientific research has been undertaken on the four natures since 1960, mainly in China and Japan. There are two main research areas on the four natures. The first is pharmacodynamic research, exploring the effect of cold and hot herbs on central nerve transmitters, sympathetic-adrenomedullary system, prostaglandin and endocrine system. The second is substantial foundation research, including chemical components and especially trace elements (5). Some innovative research was from biophysics and biochemistry to study the natures of *Radix ginseng*, Folium ginseng, Flos ginseng and *Radix quinquefolium* using a microcalorimetry method (6–8).

The effects of four natures of 60 Chinese herbs were observed on organs and tissues of IRC/F1 mice using ^{14}C-2-deoxy-glucose and autoradiography. The results showed that there were character distinctions among four natures on organs and tissues (9). Compared with the research on natures, the study on flavors has focused on the relationship between chemical component and different flavor (especially abstract flavor) and the pharmacologic actions of the main components (4,10) in China. It is interesting to note that a recent submission to *Nature* relate the pharmacologic activities of Ibuprofen and Oleocanthal to their similarities of taste. This is one of the rare scientific reports noting common pharmacologic activity for compounds with similar taste (11).

Herbs of different natures and flavors exhibit different effects and are categorized differently in terms of *yin* and *yang* (12). Chinese herbs are also mainly classified on the basis of their function in theory and in the clinical setting. For the circulation- related herbs described in this study, they are classified as (12,13): function 1— drugs whose principal effects are to stop internal and external bleeding, function 2— drugs that make free the passage of blood in the vessels, promote blood circulation and disperse blood stasis, function 3— drugs that nourish the blood and are indicated for syndromes of blood deficiency and, function 4— drugs with the effects of dispelling pathogenic heat from the blood systems. These theories are the essential basis for the analyses and clinical usage of Chinese herbs.

A recent study by Ou *et al.*(1) into the antioxidant capacities of 24 commercially available plant-based medicines inspired this study. They reported a striking relationship between *in vitro* antioxidant capacity and *yin-yang* classification of the herbs. *Yang* herbs had the lowest antioxidant values, *yin* herbs had the highest.

The utility of antioxidant therapies in many diseases is well recognized. Cellular damage arising from an imbalance between free radical generating and scavenging systems has been implicated in the pathogenesis of a wide range of disorders, including cardiovascular disease, cancer and aging (14). Many Chinese herbs have already been reported as having antioxidant effects (15,16). To further explore the antioxidant nature of Chinese herbs and to assess the relationship between antioxidant capacity and the characterization of herbs within TCM, we tested the antioxidant effect of 45 herbs extracted with water.

Methods

Chemicals and Apparatus

Trolox (6-hydroxy-2, 5, 7, 8-tetramethyl-2-carboxylicacid, a water soluble homologue of vitamine E) and fluorescein {3′, 6′-dihydroxy-spiro [isobenzofuran-1 (3H), 9′ (9H)-xanthen]-3-one disodium; FL} were obtained from Aldrich (Castle Hill, NSW, Australia). AAPH (2′-Azobis(2-amidinopropane) dihydrochloride) was purchased from Wako Pure Chemical USA (Richmond, VA, USA). The 96-well polystyrene microplates were purchased from PerkinElmer Life and Analytical Sciences. All ORAC analyses were performed on a Victor 2 plate reader (Wallac).

Samples

The samples in this study were purchased from Shanxi Provincial People's Hospital. The herbs were identified by Shanxi Provincial Medicinal Materials Supervision Bureau and the vouchered specimens were stored in sealed containers at room temperature (10–20°C). The herbs were dried under high vacuum to constant weight and ground into powder. Dried herbs (3 g) were placed in a centrifuge tube (20 ml) and mixed with water (10 ml). After 2 h at room temperature with vortex mixing and 15 min in a sonicating water bath, extracts were centrifuged (5000 g, 10 min) and the supernatants removed. Supernatants were dried under high vacuum and stored at 0°C. The dried extracts were redissolved in water at a suitable concentration for the different assays.

ORAC Assay

The assay was based on that of Ou *et al*. (17). The dried sample extracts were dissolved in water and tested at concentrations of 12.5, 25, 50 and 100 mg extract/l in triplicate. Briefly, 20 µl of sample or Trolox was incubated with 10 µl 75 nM fluorescein and 170 µl 17 mM AAPH in a total volume of 200 µl. All ORAC analyses were performed at 37°C with an excitation wavelength of 485 nm and emission wavelength of 535 nm. The area under the fluorescence decay curve for FL (AUC) was calculated by point-to-point integration. The decrease in fluorescence was followed at 1 min intervals for 35 min. The protective effect of an antioxidant was measured by comparing the AUC of the sample with that of a known antioxidant, Trolox, a water-soluble analogue of Vitamin E. The final ORAC values were expressed as micromole Trolox Equivalents per gram dried herb (µmol TE/g). Each sample was tested at four concentrations in triplicate. The results were expressed as mean (SD < 10%).

Results

ORAC Value of 45 Chinese Herbs

In this assay, 45 Chinese herbs that regulate the blood circulation were analyzed for antioxidant activity (Table 1 and Table 2). The results showed that they had a wide range of antioxidant activity. The herbs with the highest antioxidant activity were *Spatholobus suberectus* vine (1990 µmol TE/g) and *Sanguisorba officinalis* root (1940 µmol TE/g). There was a 50-fold difference in antioxidant capacity between *Spatholobus suberectus* vine (1990 µmol TE/g) and *Ziziphus jujuba* fruit (40 µmol TE/g).

Table 1. Antioxidant activity of first 10 herbs with high ORAC value and together with their classification by flavor, nature and function

Latin Binomial	ORAC (µmol TE/g)†	Flavor‡	Nature§	Function¶
Spatholobus suberectus	1990	bitter, sweet	warm	2
Sanguisorba officinalis	1940	bitter, sour	cold	1
Agrimonia pilosa	1440	bitter, sour	neutral	1
Artemisia anomala¶¶	1400	bitter	warm	2
Salvia miltiorrhiza	1320	bitter	cold	2
Sophora japonica	1300	bitter	cool	1
Nelumbo nucifera	1300	bitter	neutral	1
Crataegus pinnatifida	1240	sour, sweet	warm	2
Lycopus lucidus	1220	bitter, pungent	warm	2
Artemisia argyi	1150	pungent, bitter	warm	1

†µmol TE/g, micromoles Trolox equivalent per gram dried herb.
‡,§,¶The flavor, nature and function are according to Chinese Pharmacopoeia (2005).
¶¶The flavor, nature and function is according to Dictionary of Traditional Chinese Herb (28).

ORAC Value Comparison of Herbs with Different Natures, Flavors and Functions

Herbs with bitter flavor had a higher ORAC value than other flavors (Table 3). The average ORAC value of herbs with bitter and/or sour flavor was 856 µmol TE/g, four times higher than herbs with sweet and/or pungent flavor (213 µmol TE/g) ($P < 0.01$). Ten herbs had an

Table 2. ORAC value of 35 herbs

Latin Binomial	ORAC (µmol TE/g)	Latin Binomial	ORAC (µmol TE/g)
Platycladus orientalis	940	*Polygonum multiflorum*	790
Drynaria fortunei	700	*Paeonia lactiflora*	680
Bochmeria nivea	640	*Rubia cordifolia*	640
Gleditsia sinensis	570	*Paeonia suffruticosa*	470
Cirsium japomicum	400	*Carthamus tinctorius*	370
Cirsium setosum	370	*Paeonia lactiflora*	350
Arnebia euchroma	320	*Zingiber officinale* (carbonized†)	280
Rheum palmatum (steamed‡)	270	*Sparganium stoloniferum*	260
Vaccaria segetalis	200	*Curcuma phaeocaulis*	170
Curcuma wenyujin	140	*Ligusticum chuanxiong*	130
Imperata cylindrica	130	*Corydalis yanhusuo*	130
Typha angustifolia	120	*Bletilla striata*	100
Commiphora molmol	96	*Prunus persica*	85
Angelica sinensis	78	*Rehmannia glutinosa* (steamed‡)	77
Scrophularia ningpoensis	77	*Panax notoginseng*	75
Rehmannia glutinosa	65	*Curcuma wenyujin*	54
Boswellia carterii	49	*Cyathula officinalis*	43
Ziziphus jujuba	40		

†Carbonized medicine involves heating a drug to the point that the outer layer is burned black and the inner part brown/partly carbonized.
‡Steamed medicines are heated with vapour.

Table 3. Comparison of mean ORAC values for the different characters with which the herb may be classified based on TCM; errors are presented as SD

Medicinal characteristics			Number of herbs in group	max µmol TE/g	min µmol TE/g	mean µmol TE/g
Flavor†	bitter and/or sour	*yin*	15	1940	96	856 ± 550*
	bitter with sweet and pungent sour with sweet and pungent	*yin* within *yang* and *yang* within *yin*	17	1990	43	528 ± 558
	pungent and/or sweet	*yang*	12	640	40	213 ± 198*
Nature	cold and cool	*yin*	18	1940	54	559 ± 504
	hot and warm	*yang*	19	1990	40	558 ± 567
	neutral		8	1440	43	443 ± 540
Function	stop internal and external bleeding		15	1940	75	722 ± 568
	promote blood circulation and disperse blood stasis		19	1990	43	537 ± 584
	nourishing the blood		5	790	40	267 ± 284
	dispelling pathogenic heat from the blood systems		6	680	65	313 ± 215

†The flavor of *Arnebia euchroma* is sweet and salty; it is not included in the list.
*The mean ORAC value for bitter, sour flavor is significantly different from sweet, pungent flavor. ($P < 0.01$), Student's *t*-test.

ORAC value that was similar or higher than that of vitamin E. Figure 1 shows a breakdown of these 10 herbs into their particular flavor classifications.

Discussion

The ORAC assay has become a valuable and popular method for measuring the antioxidant or radical scavenging capacity of biological samples. A trend has been to investigate a variety of plants as new potential sources of antioxidants. Many phytochemicals have antioxidant activity and may help protect cells against the oxidative damage caused by free radicals, such as quercetin that belongs to a group of polyphenolic substances known as flavonoids and has strong antioxidant activity (18). Research into the antioxidant properties of herbs may provide information on the mechanism of action of the plant extracts, the utility of the medicines in mitigating against oxidative damage and help to identify the presence of specific antioxidant constituents. In this assay, 45 Chinese herbs that regulate blood circulation were analyzed for antioxidant activity. The results showed that they had a wide range of antioxidant activity. There was a 50-fold difference in antioxidant capacity between *Spatholobus suberectus* vine (1990 µmol TE/g) and *Ziziphus jujuba* fruit (40 µmol TE/g).

One difference between our protocol and that of Ou *et al.* (13) is that we used extracts prepared by the traditional method of water extraction while the earlier study employed 70% acetone extraction. We focused on the herb's clinical usage form, which is generally prepared by water and still most widely used at present. Another difference is that in choosing the 45 herbs we also considered their ancient pharmacologic

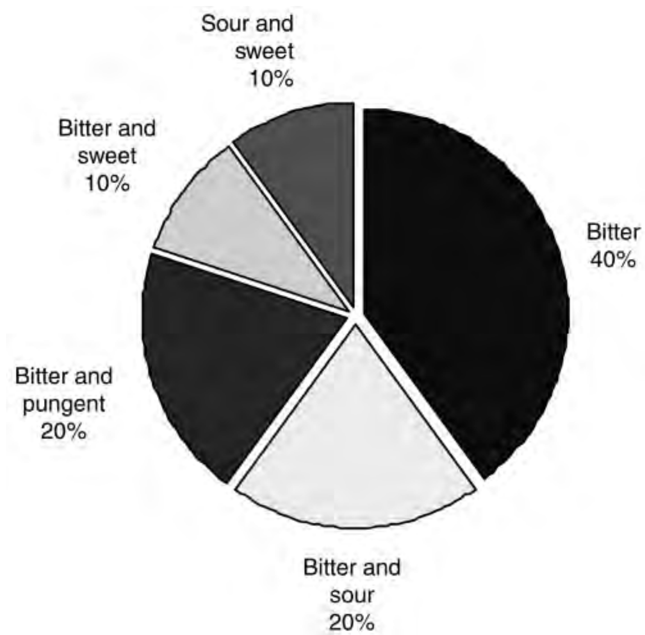

Figure 1. Comparison of the flavors of 10 herbs that had ORAC value similar to or higher than that of Vitamin E (1162 µmol TE/g). Bitter, bitter and sour belong to *yin*. Bitter and pungent, bitter and sweet, sour and sweet belong to *yin* within *yang* and *yang* within *yin*.

properties—natures, flavors and functions and the relationship of these properties with antioxidant activity. If a combination of ORAC values, natures, flavors, functions and *yin*/*yang* theory were all taken into consideration, it would assist in the selection of the best herbs to achieve optimal therapeutic activity.

Chinese herbs have their characterization based on natures and flavors. In the earliest extant medical canon in China, *Huangdi Neijing* (19), pungent flavor disperses, sweet flavor nourishes and these flavors pertain to *yang*. Sour flavor astringes, bitter flavor purges and salty flavor moistens and sour, bitter and salty flavors pertain to *yin*. For the different natures, *Huangdi Neijing* gave the following information: cold and cool natures belong to *yin* and hot and warm natures belong to *yang*. However, the *yin* and *yang* of things are relative, not absolute. Many herbs have two or more flavors and they are opposite, in this case there exists *yin* within *yang* and *yang* within *yin*. Drugs of different flavors, natures and different compositions show different pharmacologic and therapeutic actions.

In Table 3, we compared the ORAC value of herbs with different natures, flavors and functions as well as the ascribed *yin*/*yang* properties of the sub-groups. The bitter and/or sour flavors belong to *yin* character and sweet and/or pungent belong to *yang*. For the different flavors, there was a significant difference in antioxidant value. Herbs with bitter flavor have a higher ORAC value than those with pungent and/or sweet flavors. The average ORAC value of herbs with bitter and/or sour flavor is 856 μmol TE/g, four times higher than herbs with sweet and/or pungent flavor (213 μmol TE/g). The highest ORAC value in the bitter and/or sour group is 1940 μmol TE/g, three times that of the highest in the sweet and/or pungent group (640 μmol TE/g). From Figure 1, of the first ten herbs that had ORAC value similar to or higher than vitamin E (1162 μmol TE/g) (20), 60% of them have bitter and/or sour flavor (*yin*). Nine of the 10 have a bitter flavor component. Bitter flavor has the actions of drying and eliminating dampness, lowering rebellious *qi* and purging heat. Those bitter in flavors are used widely. Damp-heat syndrome is the main syndrome where bitter herbs could be used clinically.

An animal model for the study of damp-heat syndrome was developed by multiple factors (diet, climate and pathogenic biological factors) (21,22). The research showed that the activity of super-oxide dismutase (SOD) was lowered and malonyldialdehyde (MDA) increased markedly in red cells of damp-heat syndrome animals. Clinical research found an imbalance between oxidation and antioxidation of bronchitis patients with damp-heat syndrome. The antioxidant activity in the blood serum of the damp-heat syndrome group was lower than the healthy group and the non-damp-heat syndrome group (23). Of the herbs we tested, it is known that *Lycopus lucidus* is used to promote blood circulation, promote diuresis and relieve edema (edema was related to dampness in TCM theory). *Salvia miltiorrhiza* is used to promote blood circulation to remove blood stasis, cool the blood to relieve carbuncles, clear away heat from the heart and tranquilize the mind. *Sanguisorba officinalis, Sophora japonica, Nelumbo nucifera* are used to cool the blood and stop bleeding. They all had the same bitter flavor and showed high ORAC values. The mechanism of action of bitter flavor herbs in clearing heat and removing damp might have a relationship with enhanced antioxidant activity.

Phenolic or flavonoid compounds have long been recognized as potent antioxidants (24). Studies have shown that the active components of the *yin* herbs are mainly flavonoids, which are phenolic compounds with strong antioxidant activity (13). The three herbs with the highest antioxidant activity in our study, *Spatholobus suberectus*, *Sanguisorba officinalis* and *Agrimonia pilosa* were reported to contain flavonoids (25–27). Further research on compounds with antioxidant activity in the bitter flavor herbs should provide a better understanding of the ancient flavors especially abstract flavors, at a molecular level. We first consider in depth with taste and smell separately and then combine them with modern research. In smell receptors, a molecule's specific size and shape activate the receptor, while in taste receptors specific chemical reactivity may be more involved. How it works for the combination of gustation and olfaction constituting ancient flavors in modern antioxidant research might be a new point of view (11).

However, using a similar analysis we would expect the nature of cooling and function 4 (dispelling pathogenic heat from the blood systems) to also share high antioxidant properties and this was not the result obtained. Natures are also related to the *yin*/*yang* designation. In this study, the cooling/warming herbs and hence, the *yin*/*yang* designations based on these characteristics, were not different in antioxidant properties.

Overall, our study is supportive of that of Ou *et al*. and provides some insights into the four natures and five flavors, that is, bitter flavor (belonging to *yin*) had high antioxidant value. It is obvious that only one pharmacologic parameter *in vitro* from 45 herbs could not establish the antioxidant activity and relationship with their TCM characteristics conclusively and the demarcation between *yin* and *yang* is not as stark in our studies as in the Ou *et al*. publication. We attribute this to our criterion for including a medicine, being its application to circulatory conditions, while the earlier study selected plants and animals with the polar opposites of *yin* and *yang*. This support for traditional insights into medicines being consistent with mechanism of action studies is a recurring theme for natural products research and suggests biomedical research that supports the contribution and the use of these medicines.

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