

Antioxidant Activities of Some Common Ingredients of Traditional Chinese Medicine, *Angelica sinensis*, *Lycium barbarum* and *Poria cocos*

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The antioxidant activities of three popular ingredients of traditional Chinese medicine, namely *Angelica sinensis* (AS), *Lycium barbarum* (LB) and *Poria cocos* (PC) were evaluated in this study. The results showed that aqueous extracts of these crude drugs exhibited antioxidant activities in a concentration-dependent manner. All extracts displayed an inhibitory effect on FeCl₂-ascorbic acid induced lipid peroxidation in rat liver homogenate *in vitro*, with the order of activity LB > AS > PC. The tested extracts showed a superoxide anion scavenging activity ranging from 28.8% to 82.2% and anti-superoxide activity varying from 38.0% to 84.5%. Among the different extracts, LB extract exhibited the lowest IC₅₀ values (0.77–2.55 µg/mL) in all model systems tested in this study. The present study concludes that LB extract possessed the strongest inhibition on malondialdehyde formation in rat liver homogenate, and superoxide anion scavenging and anti-superoxide formation activities. These results also suggest that LB extract is a good source of antioxidant agent in the daily dietary supplement. Copyright © 2004 John Wiley & Sons, Ltd.

Keywords: *Angelica sinensis*; *Lycium barbarum*; *Poria cocos*; antioxidant activity; lipid peroxidation; anti-superoxide.

INTRODUCTION

Free radicals are known to be the main cause of aging, coronary heart disease, stroke, diabetes mellitus, rheumatism and cancer (Wong *et al.*, 1987; Halliwell, 1997; Droge, 2002). It has been reported that reactive oxygen species (ROS) such as •O₂⁻ (superoxide anion), •OH (hydroxyl radical), H₂O₂ (hydrogen peroxide) and ¹O₂ (singlet oxygen), play an important role in cellular injuries and also initiate the peroxidation of polyunsaturated fatty acids in biological membranes (Compori, 1985; Halliwell, 1997). The tissue injury caused by ROS includes DNA damage (Halliwell, 1997), protein damage (Bartold *et al.*, 1984) and oxidation of enzymes (Varani *et al.*, 1985) in the human body. Aerobic organisms are protected from oxygen toxicity by a natural antioxidant defence system involving enzymatic and non-enzymatic mechanisms (Cotgreave *et al.*, 1988; Ames *et al.*, 1993). In the enzymatic defence mechanism, such as superoxide dismutase (SOD), which catalyses the breakdown of •O₂⁻ to O₂ and H₂O₂, as well as preventing the formation of •OH (Lee *et al.*, 2003). It has been reported that non-enzymatic antioxidants such as ascorbic acid, α-

tocopherol, carotenoids, flavonoids and micronutrients such as zinc and selenium are present in notably high levels in a number of medicinal plants (Polidori *et al.*, 2001; Lin *et al.*, 2001). Natural antioxidants derived from plant products, such as herbs (Xuejiang Wang *et al.*, 2001), legumes (Lin *et al.*, 2001), tea (Roedig-Penman and Gordon, 1997) and *Panax ginseng* (Keum *et al.*, 2000) were reported to prevent oxidative stresses.

Angelica sinensis (AS), *Lycium barbarum* (LB) and *Poria cocos* (PC) are ingredients widely used in the preparation of traditional Chinese medicine in China and Taiwan. Traditionally, *A. sinensis* AS (Umbelliferae) is mainly used for treating anaemia, gynaecological disorders, hypertension, headache, asthma and rheumatism. The fruit of *L. barbarum* LB (Solanaceae) is commonly used as antipyretic, antiinflammation, pneumonia, nephritis, diuretic and antisenile agents. *P. cocos* PC (Polyporaceae) is a saprophytic fungus. Its sclerotium, commonly known as 'Hoelen', is often prescribed in combination with other crude drugs for use as a diuretic, sedative, tonic and antitumour agent (Hon *et al.*, 1990; Kaminaga *et al.*, 1996). Hot water extracts of medicinal plants are often used to prepare traditional Chinese medicated diets. However, it remains unclear if the beneficial effects of aqueous extracts of *A. sinensis*, *L. barbarum* and *P. cocos* are derived from their antioxidant properties. Therefore, the aim of present study was to evaluate the antioxidant activities of extracts of these three crude drugs prepared according to traditional practice. Its activities were also compared with α-tocopherol.

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MATERIALS AND METHODS

Plants. Samples from the roots of *Angelica sinensis* (Oliv.) Diels (AS), the fruits of *Lycium barbarum* L. (LB) and the sclerotium of *Poria cocos* (Schw.) Wolf (PC) were purchased from the Chyuan-Chang Chinese pharmacy in Tainan, Taiwan. Their authenticity was confirmed by Dr J. J. Yang, Department of Pharmacy, Chia-Nan University of Pharmacy and Science, Tainan, Taiwan.

Chemicals. L-(+)-Ascorbic acid, thiobarbituric acid, xanthine, xanthine oxidase and cytochrome c were purchased from Sigma Chemical Co. (St Louis, MO, USA). α -Tocopherol and ferrous chloride were obtained from Wako Pure Chemical Industries (Osaka, Japan). The other chemicals used were of analytical grade.

Animals. Male Wistar rats, 4–6 weeks old were obtained from the Animal Centre, National Cheng Kung University, Tainan. They were housed in an air-conditioned room with temperature maintained at $22^{\circ} \pm 3^{\circ}\text{C}$ and humidity at $55\% \pm 5\%$. Animals were fed a standard laboratory diet and tap water *ad libitum* throughout the experimental period.

Preparation of extract. One hundred grams of each sample was extracted with 1 L of boiling water for 1 h. The extract was filtered and the residue was re-extracted under the same conditions twice. The combined filtrates of each sample were evaporated to dryness under vacuum. The yield of AS, LB and PC aqueous extracts was 15.63%, 10.27% and 4.10%, respectively.

FeCl₂-ascorbic acid stimulated lipid peroxidation in rat liver homogenate. The effect of aqueous extracts on FeCl₂-ascorbic acid induced lipid peroxidation in rat liver homogenate was determined by the method of Kimuya *et al.* (1981) and Wong *et al.* (1987). A mixture containing 0.5 mL of liver homogenate, 0.1 mL of Tris-HCl buffer (pH = 7.2), 0.05 mL of 0.1 mM ascorbic acid and 0.05 mL of 4 mM FeCl₂ and 0.05 mL of various concentrations of aqueous extracts or α -tocopherol, were incubated for 1 h at 37 °C. After incubation, 0.9 mL of distilled water and 2 mL of 0.6% thiobarbituric acid were added and then shaken vigorously. The mixture was heated for 30 min in a boiling water bath (100 °C). After cooling, 5 mL *n*-butanol was added and the mixture was shaken vigorously. The *n*-butanol layer was separated by centrifugation at 3000 rpm for 10 min. The absorbance of the supernatant was read at 532 nm against a blank, which contained all the reagents except the liver homogenate. The protein content was determined according to the method described by Lowry *et al.* (1951).

Cytochrome c test. Enzyme formation of superoxide anions were assayed by the reduction of cytochrome c as method described by McCord and Fridovich (1969). Fifteen milligrams of sample was dissolved in 1 mL of distilled water and then diluted with distilled water to various concentrations (0.1 to 10.0 mg/mL). The reaction was carried out by adding 0.07 units/mL of xanthine oxidase, 100 μM of xanthine and 50 μM of cytochrome c to the samples. They were then incubated for 3 min at

room temperature, followed by spectrometric measurement at a wavelength of 550 nm.

Xanthine oxidase inhibition test. Xanthine oxidase activity was estimated by the formation of uric acid from xanthine (Chang *et al.*, 1994). Fifteen milligrams of sample was dissolved in 1 mL distilled water, followed by dilution with 50 mM KH₂PO₄ buffer (pH = 7.8) to various concentrations (0.1–10.0 mg/mL). After 100 μM of xanthine in phosphate buffer and 20 μL of xanthine oxidase (0.4 units) were added, samples were incubated for 3 min at room temperature. Superoxide formation was examined by measuring uric acid production with spectrophotometric reading at 295 nm.

Statistical analysis. The data are presented as mean \pm SD. The IC₅₀ value of each sample was calculated. The values were evaluated by one way analysis of variance (ANOVA), followed by post-hoc Duncan's multiple range tests.

RESULTS

Anti-lipid peroxidation activity

The inhibitory effect of all extracts was compared with α -tocopherol on FeCl₂-ascorbic acid induced malondialdehyde (MDA) production in rat liver homogenate *in vitro*. The results showed that the inhibitory effects of aqueous extracts on MDA formation increased with increasing concentrations of the tested crude drugs (Table 1). Extracts at concentrations of 0.5–5.0 mg/mL displayed anti-lipid peroxidation activity ranging from 20.3% to 70.1%. Significant differences ($p < 0.05$) were noted between the different concentrations of aqueous extracts of various crude drugs. At a concentration of 5 mg/mL, the formation of MDA was inhibited by 70.1%

Table 1. Inhibitory effect of different concentrations of aqueous extracts of *A. sinensis* (AS), *L. barbarum* (LB), *P. cocos* (PC) and α -tocopherol on MDA (production) induced by FeCl₂-ascorbic acid (AA) in rat liver homogenate *in vitro*

Sample	Concentration (mg/mL)	MDA ^a (nm/mg protein)	Inhibition rate (%) ^b
FeCl ₂ -AA	–	43.0 \pm 0.2	–
Control	–	13.0 \pm 0.1	–
AS (root)	0.5	36.9 \pm 0.9	20.3 ^d
LB (fruit)	0.5	36.3 \pm 0.1	22.0 ^c
PC (sclerotium)	0.5	34.1 \pm 0.4	29.7 ^b
α -Tocopherol	0.5	12.4 \pm 0.5	100.0 ^a
AS (root)	1.0	29.4 \pm 0.8	45.0 ^c
LB (fruit)	1.0	28.5 \pm 0.7	48.0 ^b
PC (sclerotium)	1.0	28.7 \pm 0.5	48.0 ^b
α -Tocopherol	1.0	9.0 \pm 0.1	100.0 ^a
AS (root)	5.0	26.0 \pm 0.1	56.7 ^c
LB (fruit)	5.0	22.1 \pm 0.3	70.1 ^b
PC (sclerotium)	5.0	26.4 \pm 0.9	55.3 ^c
α -Tocopherol	5.0	7.1 \pm 0.3	100.0 ^a

^a MDA data were presented as the mean \pm SD ($n = 5$)

^b The inhibitory rates within the column of same concentration with the different superscript letters were significantly different at $p < 0.05$ as analysed by Duncan's multiple range tests.

Table 2. Superoxide scavenger activity of different concentrations of aqueous extracts of *A. sinensis* (AS), *L. barbarum* (LB), *P. cocos* (PC) and α -tocopherol in the cytochrome c test

Sample	Concentration (mg/mL)	Inhibition rate (%) ^a
AS (root)	0.1	28.8 ± 0.5 ^d
LB (fruit)	0.1	35.1 ± 0.2 ^b
PC (sclerotium)	0.1	30.0 ± 0.4 ^c
α -Tocopherol	0.1	100.0 ± 0.1 ^a
AS (root)	1.0	46.7 ± 0.1 ^d
LB (fruit)	1.0	50.2 ± 0.7 ^b
PC (sclerotium)	1.0	48.3 ± 0.3 ^c
α -Tocopherol	1.0	100.0 ± 0.0 ^a
AS (root)	5.0	62.6 ± 0.1 ^d
LB (fruit)	5.0	66.6 ± 0.1 ^c
PC (sclerotium)	5.0	70.2 ± 0.5 ^b
α -Tocopherol	5.0	100.0 ± 0.1 ^a
AS (root)	10.0	75.4 ± 0.2 ^c
LB (fruit)	10.0	82.2 ± 0.5 ^b
PC (sclerotium)	10.0	75.6 ± 0.1 ^c
α -Tocopherol	10.0	100.0 ± 0.2 ^a

Data were presented as the percentage scavenging of free radicals, mean ± SD ($n = 3$).

^a Values within the column of same concentration with the different superscript letters were significantly different at $p < 0.05$ as analysed by Duncan's multiple range tests.

for LB, 56.7% for AS and 55.3% for PC, respectively. Among the three different extracts, LB exhibited a stronger inhibitory effect on the MDA formation than those of AS and PC extracts.

In the thiobarbituric acid test, the IC₅₀ values of the different crude drugs were in the range 2.55–4.22 mg/mL (Table 4). The LB extract showed the most potent activity, followed by AS and PC.

Free radical scavenger activity

Enzymatic formation of superoxide anions was estimated by reduction of cytochrome c. Results on the free radical scavenging activity of extracts at different concentrations are shown in Table 2. The scavenging effect on superoxide anions of the three tested extracts was found to increase with increasing concentration of crude drugs. Extracts at concentrations of 0.1–10.0 mg/mL showed scavenging activities varying from 28.8% to 82.2%.

In the cytochrome c test, the IC₅₀ value of the crude drugs was found to range from 0.95–2.69 mg/mL (Table 4), with the LB extract showing the highest scavenging effect on superoxide anions.

Table 3. Antioxidant activity of different concentrations of aqueous extracts of *A. sinensis* (AS), *L. barbarum* (LB), *P. cocos* (PC) and α -tocopherol in the xanthine oxidase inhibition test

Sample	Concentration (mg/mL)	Inhibition rate (%) ^a
AS (root)	0.1	38.0 ± 0.3 ^c
LB (fruit)	0.1	40.7 ± 0.2 ^b
PC (sclerotium)	0.1	38.5 ± 0.6 ^c
α -Tocopherol	0.1	100.0 ± 0.1 ^a
AS (root)	1.0	47.6 ± 0.5 ^c
LB (fruit)	1.0	57.8 ± 0.2 ^b
PC (sclerotium)	1.0	41.8 ± 0.7 ^d
α -Tocopherol	1.0	100.0 ± 0.1 ^a
AS (root)	5.0	60.8 ± 0.2 ^d
LB (fruit)	5.0	70.7 ± 0.3 ^b
PC (sclerotium)	5.0	67.7 ± 0.6 ^c
α -Tocopherol	5.0	100.0 ± 0.1 ^a
AS (root)	10.0	86.2 ± 0.5 ^c
LB (fruit)	10.0	88.5 ± 0.1 ^b
PC (sclerotium)	10.0	81.4 ± 0.5 ^d
α -Tocopherol	10.0	100.0 ± 0.2 ^a

Data were presented as the percentage of inhibition by the superoxide formation, mean ± SD ($n = 3$).

^a Values within the column of same concentration with the different superscript letters were significantly different at $p < 0.05$ as analysed by Duncan's multiple range tests.

Anti-superoxide anions formation

The results showed that all extracts possessed anti-superoxide activity in a concentration-dependent manner (Table 3). Extracts at concentrations of 0.1–10 mg/mL inhibited the superoxide formation with activity ranging from 38.0% to 88.5%.

The xanthine oxidase inhibition test showed that the IC₅₀ value of crude drugs was in the range 0.77–3.22 mg/mL (Table 4). The order of anti-superoxide formation activity was LB > PC > AS.

DISCUSSION

Plant tissues contain a wide variety of compounds with antioxidant activity. Phenolic compounds (flavonoids and phenolic acids), nitrogen compounds (alkaloids, chlorophyll derivatives, amino acids and amines), carotenoids, lignans and terpenes were reported to possess antioxidative activity and the ability to suppress the initiation or propagation of the chain reactions (Hall and Cuppett, 1997). The principal active constituents

Table 4. IC₅₀ values of antioxidant activities of aqueous extracts of *A. sinensis* (AS), *L. barbarum* (LB) and *P. cocos* (PC)

Sample	IC ₅₀ value (mg/mL)		
	Thiobarbituric acid test	Cytochrome c test	Xanthine oxidase inhibition test
AS (root)	3.69	2.69	3.22
LB (fruit)	2.55	0.95	0.77
PC (sclerotium)	4.22	2.03	2.90

isolated from the roots of AS were ferulic acid and ligustilide (Wang *et al.*, 1999). Polysaccharide and triterpenes are the two most important components of PC. Flavonoids and betaine are reported to be the main antioxidative compounds of LB fruits (Huang *et al.*, 1998; Ren *et al.*, 1995). In this study, all extracts showed antioxidant activities, however, the magnitude of antioxidative potency varied with the type of extract. This could be due to the difference in concentrations and the type of antioxidative compounds present in these extracts.

Oxidative stress is associated with the peroxidation of cellular lipids, which is determined by measurement of TBA-reactive substances. Lipid peroxidation in biological systems has long been thought to be a toxicological phenomenon, resulting in pathological consequences (Hochstein and Jain, 1981). The rat liver homogenate was induced with FeCl₂-ascorbic acid for nonenzymatic lipid peroxidation. Fe²⁺ and ascorbic acid stimulated lipid peroxidation in rat liver microsomes and mitochondria (Kimuya *et al.*, 1981). Malondialdehyde (MDA) is very reactive and takes part in cross-linking with DNA and proteins, and also damages liver cells (Kubow, 1990). In the present study, LB was found to be most effective in preventing lipid peroxidation. In China and Taiwan, crude drugs have long been used to enhance health and to prevent diseases. Based on the results of this study, the beneficial effects of certain folk medicines could be explained, in part, by their antioxidative activities, which are able to act against free radical damage to cell membranes through reducing the level of lipid peroxides.

Xanthine oxidase is a flavoprotein, which catalyses the oxidation of hypoxanthine to xanthine and generates superoxide and uric acid (Chiang and Chen, 1993). Studies have shown that xanthine oxidase inhibitors may be useful for the treatment of hepatic disease and gout, which is caused by the generation of uric acid and superoxide anion radicals (Lin *et al.*, 2000). Xanthine oxidase-derived superoxide anion has been linked to post-ischaemic tissue injury and oedema (McCord and Fridovich 1969; Hearse *et al.*, 1986). Inhibition of superoxide anion regeneration by the enzymatic pathway would be beneficial in ischaemia and oedema. As shown in Table 2, the superoxide scavenging activity of the tested extracts was in the order of LB > PC > AS, with a trend of concentration-dependent effect noted. The LB extract showed the most potent scavenging effect on superoxide anions, suggesting its potential for use in the preparation of beverages or Chinese medicated diets for daily consumption.

In conclusion, the present study demonstrated that the aqueous extracts of AS, LB and PC possessed a different magnitude of antioxidant activities. However, the strongest antioxidant activities were exhibited by the LB extract. The results also reveal that the fruits of LB possessed potent anti-lipid peroxidation, superoxide anion scavenging and anti-superoxide formation activities. Therefore, LB could be a potential antioxidant for use in retarding the aging process and preventing diseases caused by ROS. This observation also suggests that the therapeutic benefits of LB prepared in traditional Chinese medicine could, in part, be contributed by its antioxidant activities.

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