Biol Res 40: 13-21, 2007

Antioxidant activity of extract from *Polygonum cuspidatum*

CHIN-YUAN HSU*, YU-PEI CHAN and JELI CHANG

Department of Life Science, Chang Gung University, Tao-Yuan, Taiwan

ABSTRACT

Numerous diseases are induced by free radicals via lipid peroxidation, protein peroxidation and DNA damage. It has been known that a variety of plant extracts have antioxidant activities to scavenge free radicals. Whether *Polygonum cuspidatum* Sieb. et Zucc has antioxidant activity is unknown. In this study, dried roots of *Polygonum cuspidatum* were extracted by ethanol and the extract was lyophilized. Free radical scavenging assays, superoxide radical scavenging assays, lipid peroxidation assays and hydroxyl radical-induced DNA strand scission assays were employed to study antioxidant activities. The results indicate that the IC50 value of *Polygonum cuspidatum* extract is 110 μg/ml in free radical scavenging assays, 3.2 μg/ml in superoxide radical scavenging assays, and 8 μg/ml in lipid peroxidation assays, respectively. Furthermore, *Polygonum cuspidatum* extract has DNA protective effect in hydroxyl radical-induced DNA strand scission assays. The total phenolics and flavonoid content of extract is 641.1 ± 42.6 mg/g and 62.3 ± 6.0 mg/g. The results indicate that *Polygonum cuspidatum* extract clearly has antioxidant effects.

Key terms: antioxidant activity, free radicals, phenolics, lipid peroxidation, DNA damage *Polygonum cuspidatum* Sieb. et Zucc

INTRODUCTION

Ultraviolet light, ionizing radiation, chemical reactions and metabolic processes can induce the production of reactive oxygen species in the cells. These reactive oxygen species cause lipid peroxidation, protein peroxidation, DNA damage and cellular degeneration in the cells. The accumulation of these damages can induce numerous diseases including cardiovascular disease, ageing, cancer, inflammatory diseases and a variety of other disorders (1, 2, 3, 4, 5, 6). Reactive oxygen species primarily consist of superoxide radical anion ($\cdot O_2^-$), hydroxyl radicals ($\cdot OH$), singlet oxygen ($^1O_2$) and hydrogen peroxide (H_2O_2). The damaging action of the hydroxyl radical is the strongest among free radicals. In cellular oxidation reactions, superoxide radical is normally formed first. It produces other kinds of cell-damaging free radicals and oxidizing agents. In this way, its oxidizing effects can be magnified (7).

Phenolics have been known to possess a capacity to scavenge free radicals. They are commonly found in both edible and nonedible plants, and have multiple biological effects, including antioxidant activity (8, 9). The antioxidant activity of phenolics is principally due to their redox properties, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers. In addition, they have a metal chelation potential (10). Phenolics, such as flavonoids, phenolic acids, stilbenes, lignans, lignin and tannins, are especially common in leaves, flowering tissues, and woody parts such as stems and

Abbreviation: DPPH; 1,1-Diphenyl-2-picrylhydrazyl, NBT; nitroblue tetrazolium chloride, TBA; 2-thiobarbituric acid.

* Correspondence and reprints: Dr. Chin-Yuan Hsu, Department of Life Science, Chang Gung University, 259, Wen-Hwa 1st Road, Kwei-Shan, Tao-Yuan 333, Taiwan, Tel: 886-3-2118800 ext 3402, E-mail: hsu @mail.cgu.edu.tw

Received: May 1, 2006. In revised form: July 31, 2006. Accepted: August 8, 2006

barks (11). Larson has proposed that they play an important preventive role in the development of cancer, heart disease and ageing-related diseases.

The importance of the antioxidant constituents of plant materials in the maintenance of health and protection from ageing-related diseases has intrigued scientist for a long time. We have screened the antioxidant activity of a variety of wild plants and other substances used in traditional Oriental medicine, using 1,1-Diphenyl-2-picrylhydrazyl (DPPH) free radical scavenging assays. The results indicate that roots of *Polygonum cuspidatum* Sieb. et Zucc (Polygonaceae) exhibits a DPPH free radical scavenging effect. *Polygonum cuspidatum* has been traditionally used for treatment of various inflammatory diseases, hepatitis, tumors and diarrhea (12). Recently, its extracts have been reported to possess the antiviral activity against hepatitis B virus (13), have potent estrogenic activities (14), inhibit bacterial DNA primase (15), and inhibit acyl-coenzyme A-cholesterol acyltransferase activity (16). Nevertheless, it is unknown whether *Polygonum cuspidatum* Sieb. et Zucc has antioxidant activity on superoxide radical scavenging, lipid peroxidation and DNA damage. The content of total phenolics and flavonoids of *Polygonum cuspidatum* Sieb. et Zucc has not been described. In the present study, we have studied the antioxidant properties of *Polygonum cuspidatum*.

METHODS

Chemicals

1,1-Diphenyl-2-picrylhydrazyl (DPPH), nitroblue tetrazolium chloride (NBT), 2-thiobarbituric acid (TBA), ΦX174 RF1 supercoiled DNA, Folin-Ciocalteu reagent, L-ascorbic acid and (+)-catechin were purchased from Sigma Chemical Co. The other chemicals and solvents used in this experiment were of the highest quality available. Dried roots of *Polygonum cuspidatum* were purchased from a drug store in Taiwan.

Preparation of plant extract

The plant extract was prepared as previously described in Chang et al (17) and Hsu (18). Dried powder of *Polygonum cuspidatum* (5 g) were extracted in 50 ml 50% ethanol solution at 25°C for 30 min with shaking. The extract was centrifuged at 15000 rpm for 3 min and supernatant was collected. The supernatant was concentrated in a rotary evaporator and then lyophilized.

DPPH assay

1 mg extract powder was dissolved in 1 ml of 50% ethanol solution to obtain 1000 μg/ml sample solution. 1000 μg/ml solutions were series diluted into 1 μg/ml, 5 μg/ml, 10 μg/ml, 20 μg/ml, 50 μg/ml, 100 μg/ml, 200 μg/ml, 500 μg/ml and 1000 μg/ml with 50% ethanol. In each reaction, the solutions were mixed with 1 ml of 0.1 mM 1,1-Diphenyl-2-picrylhydrazyl (DPPH), 0.45 ml of 50 mM Tris-HCl buffer (pH 7.4) and 0.05 ml samples at room temperature for 30 min. 50% ethanol solution was used as negative control. The reduction of the DPPH free radical was measured by reading the absorbance at 517 nm. DPPH is a purple-colored stable free radical; when reduced it becomes the yellow-colored diphenylpicrylhydrazine. L-Ascorbic acid and (+)-catechin were used as positive controls. The inhibition ratio (percentage) was calculated as % of inhibition = [(absorbance of control – absorbance of test sample)/absorbance of control] x 100%. The antioxidant activity of each sample was expressed in terms of IC50 (micromolar concentration required to inhibit DPPH radical formation by 50%), calculated from the inhibition curve (18, 19, 20, 21).

NBT (superoxide scavenging) assay

The superoxide anion radical scavenging activity was assayed as described by Liu and Ng (7). Superoxide radicals were generated in 3.0 ml of Tris-HCl buffer (16 mM, pH 8.0), which contained 78 μM β-nicotinamide adenine dinucleotide (reduced form, NADH), 50 μM nitroblue tetrazolium (NBT), 10 μM phenazin methosulfate

(PMS), and test samples in 50% ethanol solution (final concentrations were 1, 5, 10, 20, 50 and 100 μg/ml, respectively). The color reaction of superoxide radicals and NBT was detected at OD 560 nm. (+)-catechin was used as positive controls. The inhibition ratio (%) was calculated as % of inhibition = [(absorbance of control – absorbance of test sample)/absorbance of control] x 100%.

Lipid peroxidation assay

The brains of young adult male Balb/c mice were dissected and homogenized with a homogenizer in ice-cold Tris-HCl buffer (20 mM, pH 7.4) to produce a 1/10 homogenate. The homogenate was centrifuged at 12000g for 15 min at 4ºC, and the supernatant was used for *in vitro* lipid peroxidation assay. An aliquot of liposome (1 ml) was incubated with the test samples (final concentrations were 1, 5, 10, 20, 50 and 100 μg/ml, respectively) in the presence of 10 mM FeSO4 and 0.1 mM ascorbic acid at 37ºC for 1 h. The reaction was ended by the addition of 1.0 ml of trichloroacetic acid (TCA; 28%, w/v) and 1.5 ml of TBA (1%, w/v), followed by heating at 100ºC for 15 min. The absorbance of the malondialdehyde (MDA)-TBA complex was measured at 532 nm. (+)-catechin was used as positive controls. The inhibition ratio (%) was calculated as % of inhibition = [(absorbance of control – absorbance of test sample)/absorbance of control] x 100% (17).

DNA strand scission assay

The assay was carried out as described previously by Keum et al. with minor modifications (22). The reaction mixture (30 ml) contained 10 mM Tris-HCl, 1 mM EDTA buffer (pH 8.0), ΦX174 RF1 supercoiled DNA (0.6 μg) and H_2O_2 (0.04M). Additionally, different amounts of extract of *Polygonum cuspidatum* (to get final concentrations of 10, 100, 1000, 5000 and 10000 μg/ml) were added prior to H_2O_2 addition. Hydroxyl radicals were generated by irradiation of the reaction mixtures at a distance of 5 cm with a 12 W UV lamp (short wave). The mixture was incubated at room temperature for 2 min and the reaction was ended by addition of loading buffer (0.25% bromophenol blue tracking dye and 40% sucrose). The mixtures were analyzed by 0.8% submarine agarose gel electrophoresis (50eV, 1.5 h) as described by Chang et al (17).

Total flavonoid of determination

Samples (1 mg) were added in 1ml of 80% ethanol. An aliquot of 0.5 ml was added to test tubes containing 0.1 ml of 10% aluminum nitrate, 0.1 ml of 1 M potassium acetate and 4.3 ml of 80% ethanol. The absorbance of the supernatant was measured at 415 nm after 40 min at room temperature. Total flavonoid concentration was calculated using quercetin as standard. (23).

Total phenols determination

Total phenolics content was determined according to the Folin-Ciocalteu method (24), using gallic acid as standards. Extract powders (1 mg) were dissolved in 1 ml 50% methanol solution. Extract solution (0.5 ml) was mixed with 0.5 ml of 50% Folin-Ciocalteu reagent. After of 2-5 min, 1.0 ml of 20% Na_2CO_3 was added to the mixture and incubated for 10 min at room for temperature. The mixture was centrifuged at 150 g for 8 min and the absorbance of the supernatant was measured at 730 nm. The total phenolic content was expressed as gallic acid equivalents (GAE) in milligrams per gram sample (17).

RESULTS

The results showed that the IC50 values (the concentration required to inhibit radical formation by 50%) of *Polygonum cuspidatum* extract were about 110 μg/ml (Fig 1). The IC_{50} values of (+)-catechin and ascorbic acid were about 35 μg/ml and about 50 μg/ml, respectively. Compared to (+)-catechin and L-ascorbic acid, they exhibited a similar curve of antioxidant activity. However, the IC_{50} value of *Polygonum*

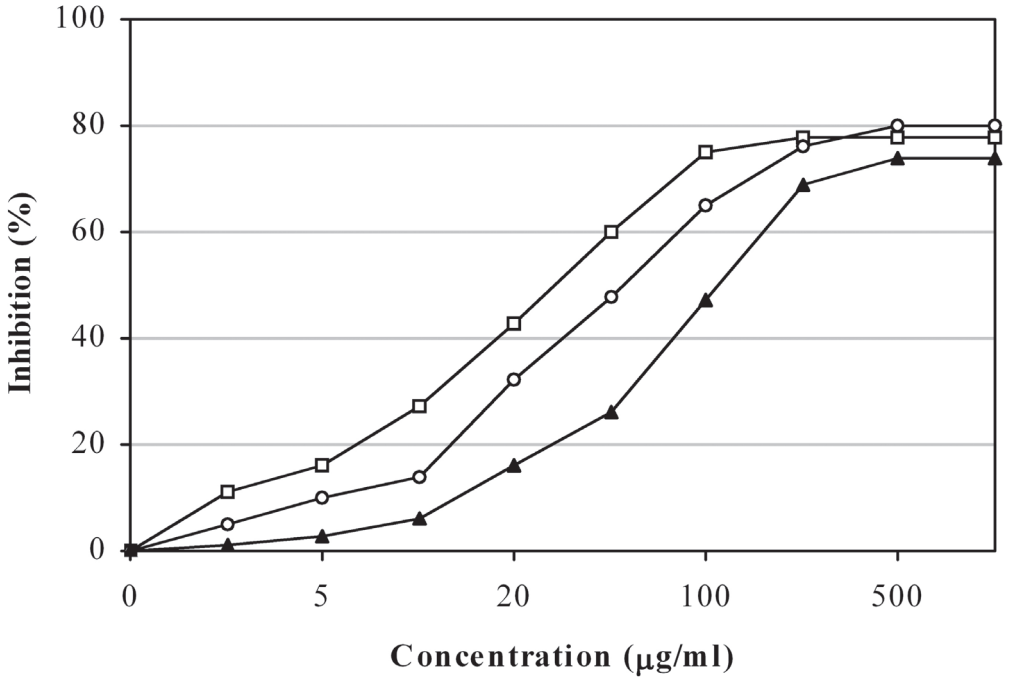

Figure 1: Free radical scavenging of *Polygonum cuspidatum* extract by the DPPH assay: (◊) (+)-catechin; (○) ascorbic acid; (▲) *Polygonum cuspidatum* extract. Results are mean ± SD (N=5).

cuspidatum extract was lower than that of (+)-catechin and L-ascorbic acid. This result demonstrated that *Polygonum cuspidatum* extract has inhibitory activity against the DPPH radical.

The figure 2 shows the superoxide scavenging activity of *Polygonum cuspidatum* extract with (+)-catechin as control. The results showed that the IC_{50} value of *Polygonum cuspidatum* was about 3.2 μg/ml, while the IC_{50} value of (+)-catechin was about 40 μg/ml. Almost all superoxide radicals were inhibited by 20 μg/ml *Polygonum cuspidatum* extract. The superoxide scavenging activity of *Polygonum cuspidatum* extract was remarkably higher than that of (+)-catechin. Therefore, *Polygonum cuspidatum* seems to be a potential source of superoxide radical scavenging. This result demonstrates that *Polygonum cuspidatum* extract has a significant superoxide scavenging activity.

The lipid peroxidation suppressing activity of *Polygonum cuspidatum* extract is shown in the Figure 3, with (+)-catechin as control. The result shows that the IC_{50} values of *Polygonum cuspidatum* extract were about 8 μg/ml (Fig. 3). The IC_{50} value of (+)-catechin was about 17 μg/ml. The lipid peroxidation suppressing activity of *Polygonum cuspidatum* extract was higher than that of (+)-catechin. Therefore, *Polygonum cuspidatum* seems to be a potential lipid peroxidation suppressor. This result demonstrated that *Polygonum cuspidatum* extract has a significant lipid peroxidation suppressing activity.

The protective effect of DNA of *Polygonum cuspidatum* extracts is shown in the Figure 4, with ΦX174 RF1 supercoiled DNA as control (lane 1). UV illumination alone did not cause DNA strand cleavage (lane 2). The treatment of supercoiled DNA with UV plus H_2O_2 led to the conversion of the DNA to open circular form (lane 3). High concentration of *Polygonum cuspidatum* extract led to the maintenance of the DNA to super coiled form (lanes 6-8), showing almost complete protection at a dose of 5000 μg/ml. This protective effect of DNA exhibits dose-dependence. This result reveals a DNA protective activity from oxidative stress of *Polygonum cuspidatum*.

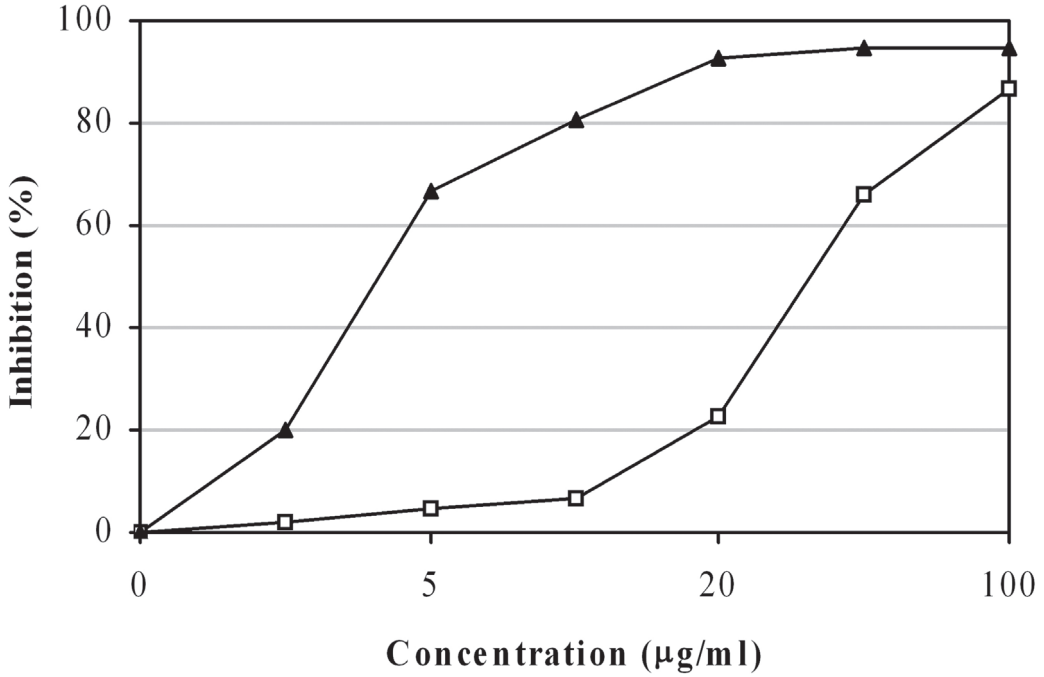

Figure 2: Superoxide scavenging of *Polygonum cuspidatum* extract by the NBT assay: (◊) (+)-catechin; (▲) *Polygonum cuspidatum* extract. Results are mean ± SD (N =5).

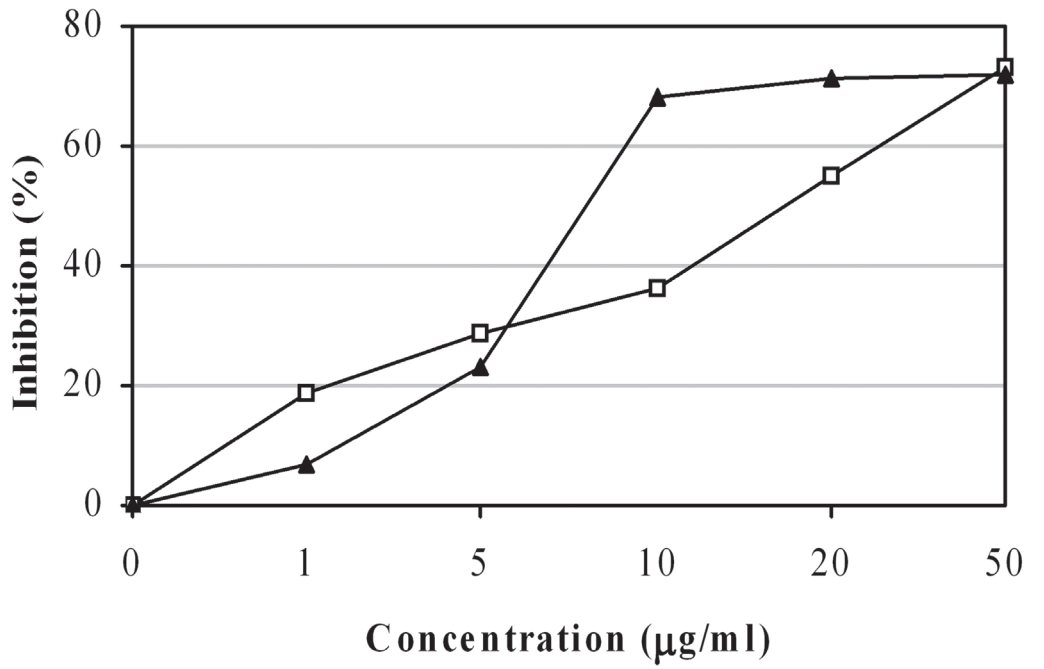

Figure 3: Effects of *Polygonum cuspidatum* extract on both ferric ion and ascorbic acid induced lipid peroxidation on mouse brain homogenates: (◊) (+)-catechin; (▲) *Polygonum cuspidatum* extract. Results are mean ± SD (N =5).

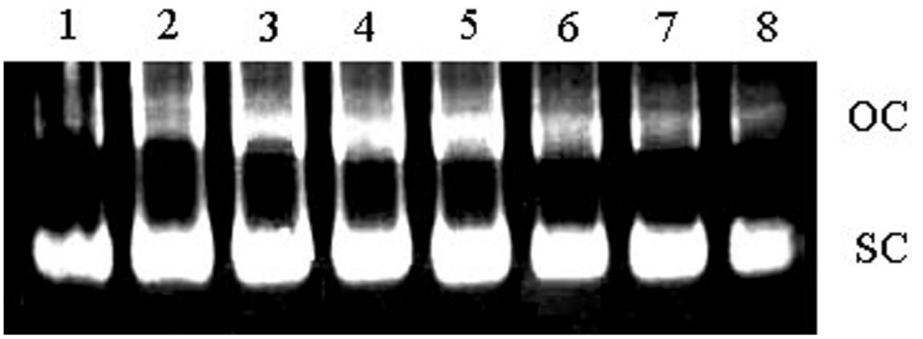

Figure 4: Protection effect of *Polygonum cuspidatum* extract on DNA strand scission induced by H_2O_2 and UV. ΦX174 RF1 supercoiled DNA as control (lane 1), ΦX174 RF1 supercoiled DNA was exposed to UV alone (lane 2), UV plus H_2O_2 (lane 3), or plus H_2O_2 in the presence of final concentration of 10 μg/ml (lane 4), 100 μg/ml (lane 5), 1000 μg/ml (lane 6), 5000 μg/ml (lane 7), 10,000 μg/ml (lane 8) of *Polygonum cuspidatum* extract. Lane 1 represents native ΦX174 RF1 supercoiled DNA without any treatment. OC: Open circular; SC: Supercoiled.

Plant phenolics are widely distributed in the tissues of plants as well as play a pivotal role in the highly effective free radical scavengers and antioxidant activity. The phenolic and flavonoid total contents in *Polygonum cuspidatum* extract were 641.1 ± 42.6 mg/g and 62.3 ± 6 mg/g, respectively (Table 1). These results imply that *Polygonum cuspidatum* extract contains a high quantity of phenolics and flavonoids.

DISCUSSION

Antioxidant activity of *Polygonum cuspidatum* extract has been revealed by free radical scavenging assays, superoxide radical scavenging assays, lipid peroxidation assays and hydroxyl radical-induced DNA strand scission assays. In addition, *Polygonum cuspidatum* extract has high phenolic and flavonoid contents. This study shows that *Polygonum cuspidatum* extract clearly has antioxidant effects.

DPPH is a stable radical that has been widely utilized to appraise the antioxidant activity of various natural products (25). In this study, DPPH scavenging activity has been found in *Polygonum cuspidatum* extract. Hsu et al (18) described 80% as maximum inhibition of (+)-catechin, ascorbic acid and *Polygonum aviculare* L. In this paper we have described 75% as maximum inhibition for *Polygonum cuspidatum*. The maximum inhibition concentration described for *Polygonum aviculare* L. and (+)-catechin is approximately 100 μg/ml (18) and 200 μg/ml for ascorbic acid. For *Polygonum cuspidatum*, we have described as maximum inhibition concentration approximately 110 μg/ml. The inhibitory curve of DPPH scavenging activity of *Polygonum cuspidatum* is similar to that of *Acacia confusa* (17), Cat's claw (*Uncaria tomentosa*) (26), *Anthriscus cerefolium* (19) and *Polygonum aviculare* L. (18). However, the IC_{50} value of *Polygonum cuspidatum* (110 μg/ml) is less than that of *Acacia confusa* (5 μg/ml), Cat's claw (*Uncaria tomentosa*) (18 μg/ml), *Anthriscus cerefolium* (45 μg/ml) (17, 19, 26) and *Polygonum aviculare* L. (50 μg/ml) (18). Nevertheless, *Polygonum cuspidatum* extract is still a source of natural antioxidants.

In cellular oxidation reactions, superoxide radicals are normally formed first, and their effects can be magnified because they produce other kinds of free radicals and oxidizing agents (7). Additionally, xanthine oxidase is one of the main enzymatic sources of those reactive oxygen species *in vivo*. In the current study, superoxide radicals scavenging property has been found in *Polygonum cuspidatum* extract. The IC_{50} value of *Polygonum cuspidatum* extract is about 3.2 μg/ml, whereas the IC_{50} value of (+)-catechin is about 40 μg/ml. The maximum inhibitory

TABLE 1

Total phenolic content and total flavonoid content of ethanolic extract from *Polygonum cuspidatum*

Extract	Total phenolic (mg of GAE/g)	Total flavonoid (mg/g of sample)
Polygonum cuspidatum	641.1 ± 42.6 (N=8)	62.3 ± 6.0 (N=10)

Total phenolics are expressed as gallic acid equivalent (GAE).
Total flavonoids are expressed as mg of total flavonoid content/g of samples based on quercetin as standard.
Values represent mean ± S.D.

effect of *Polygonum cuspidatum* is about 100%. The maximum inhibition concentration of *Polygonum cuspidatum* is approximately 20 μg/ml. However, the maximum inhibition concentration of (+)-catechin is higher than 100 μg/ml. It is noteworthy that the superoxide scavenging activity of *Polygonum cuspidatum* extract is superior to that of (+)-catechin. The IC_{50} value of *Polygonum cuspidatum* (3.2 μg/ml) is larger than that of *Paeonia suffruticosa* (50 μg/ml) (7), but less than *Polygonum aviculare* L. (0.8 μg/ml) (18). These results show that *Polygonum cuspidatum* is an important source of superoxide radicals scavenging.

In this study, lipid peroxidation of mouse brain homogenates was induced by ferric ion plus ascorbic acid. Lipid peroxidation scavenging activity has been found in *Polygonum cuspidatum* extract. The IC_{50} values of *Polygonum cuspidatum* extract were about 8 μg/ml. The IC_{50} value of (+)-catechin was about 17 μg/ml. The maximum inhibitory effect of *Polygonum cuspidatum* was about 75%. The maximum inhibition concentration of *Polygonum cuspidatum* was approximately 10 μg/ml. The maximum inhibition concentration of (+)-catechin was approximately 50 μg/ml. The inhibitory effect of *Polygonum cuspidatum* was higher than (+)-catechin. It is also higher than described previously for *Polygonum aviculare* L. (16 μg/ml) (18). This result indicates that *Polygonum cuspidatum* extract is a good source of lipid peroxidation scavenging.

The cellular damage resulting from hydroxyl radicals is strongest among free radicals. Hydroxyl radicals can be generated by biochemical reaction. Superoxide radical is converted by superoxide dismutase to hydrogen peroxide, which can subsequently produce extremely reactive hydroxyl radicals in the presence of transition metal ions such as iron and copper or by UV photolysis. Hydroxyl radicals can attack DNA to cause strand scission. Incubation of ΦX174 RF1 supercoiled DNA with H_2O_2 and then UV radiation resulted in complete conversion of supercoiled DNA to the open circular form. In this study, the administration of *Polygonum cuspidatum* extract to the reaction mixture substantially decreased the DNA strand scission induced by both H_2O_2 and UV radiation specially at high concentration. It shows a dose-dependent protection of DNA under oxidative stress. Nearly complete protection was found at a dose of 5000 μg/ml. However, the effect of DNA protection of *Polygonum cuspidatum* was lower than those described by *Acacia confusa* (1000 μg/ml) (17) and *Polygonum aviculare* L. (1000 μg/ml) (18). These results reveal that *Polygonum cuspidatum* extract is a DNA protector.

Phenolics are found in large quantities in the plant kingdom, and they have been proposed to have multiple biological functions, including antioxidant activity (27, 28, 29). Our results showed that *Polygonum cuspidatum* extract contains 641.1 ± 42.6 mg/g phenolics. The *Polygonum cuspidatum* extract contained a higher amount of phenolics than the bark (470.6 ± 43.9 mg/g phenolics) and heartwood extracts (529.7 ± 14.4 mg/g

phenolics) of *Acacia confusa* based on Folin-Ciocalteu procedures (17). However, the phenolics concentration of *Polygonum cuspidatum* extract was lower than that described for *Polygonum aviculare* L. extract (677.4 ± 62.7 mg/g phenolics). Nevertheless, *Polygonum cuspidatum* is a significant source of phenolics. The results in this study suggest that the effectiveness of the antioxidant activity of *Polygonum cuspidatum* extract is probably related to their phenolic contents, and the observed antioxidant activities of the extract may be due to the hydroxyl groups in phenolics (30). A similar finding has been demonstrated in the plant extracts of *Eucommia ulmoides* (Du-zhong), *Acacia confusa* and *Polygonum aviculare* L., where the phenolics concentration have correlated with their antioxidant activities (17, 18, 31). The flavonoid concentration in *Polygonum cuspidatum* was 62.3 ± 6 mg/g, lower than *Polygonum aviculare* L. (112.7 ± 13 mg/g), but higher than propolis (23).

The higher content of total phenolics and flavonoids in *Polygonum cuspidatum* have been correlated with hepatitis B virus inhibition (13), bacterial DNA primase inhibition (15), acyl-coenzyme A acyltransferase activity (16), inhibition of copper catalyzed oxidation for low-density lipoprotein (32), inhibition of platelet clotting and arachidonate metabolism, liver injury from peroxidized oil (33), having cancer-chemopreventive activities (34), and estrogenic activities (14, 35). Taken together, this study indicates that *Polygonum cuspidatum* extract clearly has antioxidant effects.

ACKNOWLEDGEMENTS

This work was supported by NSC 94-2311-B-182-008 grants from the National Science Council, R. O. C.

REFERENCES

1. BAUEROVA K, BEZEK A (1999) A role of reactive oxygen and nitrogen species in pathogenesis of rheumatoid disease. Gen Physiol Biophys 18: 15-20
2. FINKEL T, HOLBROOK NJ (2000) Oxidants, oxidative stress and biology of ageing. Nature 408: 239-2479
3. HALLIWELL B, GUTTERIDGE JMC, CROSS CE (1992) Free-radicals, antioxidants and human diseases: Where are we now? J Lab Clin Med 119: 598-620
4. KNIGHT JA (1995) Diseases related to oxygen-derived free radicals. Ann Clin Lab Sci 25: 111-121
5. PETRONE WF, ENGLISH DK, WONG K, MCCORD JM (1980) Free-radicals and inflammation: superoxide dependent activation of a neutrophil activating factor in plasma. Proc Natl Acad Sci USA 77: 1159-1163
6. VISIOLI F, KEAREY JF, HALLIWELL B (2000) Antioxidants and cardiovascular diseases: panaceas or tonics for tired sheep? Cardiovasc Res 47: 409
7. LIU F, NG TB (2000) Antioxidative and free radical scavenging activities of selected medicinal herbs. Life Sci 66: 725-735
8. KÄHKÖNEN MP, HOPIA AI, VUORELA HJ, RAUUHA JP, PIHLAJA K, KUJALA TS, HEINONEN M (1999) Antioxidant activity of plant extracts containing phenolic compounds. J Agric Food Chem 47: 3954-3962
9. VALENZUELA A, SANHUEZA J, NIETO S (2003) Cholesterol oxidation: Health hazard and the role of antioxidants in prevention. Biol Res 36: 291-302
10. RICE-EVANS CA, MILLER NJ, PAGANGA G (1996) Structure-antioxidant activity relationships of flavonoids and phenolic acids. Free Radical Biol Med 20: 933-956
11. LARSON RA (1988) The antioxidants of higher plants. Phytochemistry 27: 969-978
12. CHOI J, CONRAD CC, MALAKOWSKY CA, TALENT JM, YUAN CS,GRACY RW (2002) Flavones from *Scutellaria baicalensis* Georgi attenuate apoptosis and protein oxidation in neuronal cell lines. Biochim. Biophys. Acta 1571: 201-210
13. CHANG J-S, LIU H-W, WANG K-C, CHEN M-C, CHIANG L-C, HUA Y-C, LIN C-C (2005) Ethanol extract of *Polygonum cuspidatum* inhibits hepatitis B virus in a stable HBV-producing cell line. Antiviral Res 66: 29-34
14. MATSUDA H, SHIMODA H, MORIKAWA T, YOSHIKAWA M (2001) Phytoestrogens from the roots of *Polygonum cuspidatum* (Polygonaceae): structure-requirement of hydroxyanthraquinones for estrogenic activity. Bioorg Med Chem Lett 11: 1839-1842
15. HEGDE VR, PU H, PATEL M, BLACK T, SORIANO A, ZHAO W, GULLO VP, CHAN T-M (2004) Two new bacterial DNA primase inhibitors from the plant *Polygonum cuspidatum*. Bioorg Med Chem Lett 14: 2275-2277
16. PARK C-S, LEE Y-C, KIM J-D, KIM H-M, KIM C-H (2004) Inhibitory effects of *Polygonum cuspidatum* water extract (PCWE) and its component rasveratrol on acyl-coenzyme A-cholesterol acyltransferase activity for cholesteryl ester synthesis in HepG2 cells. Vascul Pharmacol 40: 279-284
17. CHANG S-T, WU J-H, WANG S-Y, KANG P-L, YANG N-S, SHYUR L-F (2001) Antioxidant activity of extracts from *Acacia confuse* bark and heartwood. J Agric Food Chem 49: 3420-3424
18. HSU C-Y (2006) Antioxidant activity of extract from *Polygonum aviculare* L. Biol Res 39: 281-288
19. FEJES S, BLÁZOVICS A, LUGASI A, LEMBERKOVICS E, PETRI G, KÉRY A (2000) In vitro antioxidant activity of *Anthriscus cerefolium* L. (Hoffm.) extracts. J Ethnopharmacol 69: 259-265
20. GYAMFI MA, YONAMINE M, ANIYA Y (1999) Free-radical scavenging action of medicinal herbs from

Ghana *Thonningia sanguinea* on experimentally-induced liver injuries. Gen Pharmacol 32: 661-667
21. YOKOZAWA T, CHEN CP, DONG E, TANAKA T, NONAKA GI, NISHIOKA I (1998) Study on the inhibitory effect of tannins and flavonoids against the 1, 1-diphenyl-2-picrylhydrazyl radicals. Biochem Pharmacol 56: 213-222
22. KEUM YS, PARK KK, LEE JM, CHUN KS, PARK JH, LEE SK, KWON H, SURH YJ (2000) Antioxidant and anti-tumor promoting activities of the methanol extract of heat-processed ginseng. Cancer Lett 150: 41-48
23. NIEVA MORENO MI, ISLA MI, SAMPIETRO AR, VATTUONE MA (2000) Comparison of the free radical-scavenging activity of propolis from several regions of Argentina. J Ethnopharmacol 71: 109-114
24. ROBAK J, GRYGLEWSKI RJ (1996) Bioactivity of flavonoids. Polish J Ethnopharmacol 48: 555-564
25. HU C, KITTS DD (2000) Studies on the antioxidant activity of *Echinacea* root extract. J Agric Food Chem 48: 1466-1472
26. SANDOVAL M, CHARBONNET RM, OKUHAMA NN, ROBERTS J, KRENOVA Z, TRENTACOSTI AM, MILLER MJ (2000) Cat's claw inhibits TNFalpha production and scavenges free radicals: Role in cytoprotection. Free Radical Biol Med 29: 71-78
27. RICARDO DA SILVA JM, DARMIN N, FERNANDEZ Y, MITJAVILA S (1991) Oxygen free radical scavenger capacity in aqueous models of different procyanidins from grape seeds. J Agric Food Chem 39: 1549-1552
28. SATO M, RAMARATHNAM N, SUZUKI Y, OHKUBO T, TAKEUCHI M, OCHI H (1996) Varietal differences in the phenolic content and superoxide radical scavenging potential of wines from different sources. J Agric Food Chem 44: 37-41
29. KAPLAN M, AVIRAM M (2004) Red wine administration to apolipoprotein E-deficient mice reduces their macrophage-derived extracellular matrix atherogenic properties. Biol Res 37: 239-245
30. HATANO T, EDAMATSU R, HIRAMATSU M, MORI A, FUJITA Y, YASUHARA T, YOSHIDA T, OKUDA T (1989) Effects of interaction of tannins with co-existing substances. VI. Effects of tannins and related polyphenols on superoxide anion radical and on DPPH radical. Chem Pharm Bull 37: 2016-2021
31. YEN G-C, HSIEH C-L (1998) Antioxidant activity of extracts from Du-zhong (*Eucommia ulmoides*) toward various lipid peroxidation models in vitro. J Agric Food Chem 46: 3952-3957
32. WATERHOUSE AL, LAMUELA-RAVENTOS RM (1994) The occurrence of piceid, a stilbene glucoside, in grape berries. Phytochemistry 37: 571-573
33. JANG M, CAI L, UDEANI GO, SLOWING KV, THOMAS CF, BEECHER CWW, FONG HHS, FARNSWORTH NR, KINGHORN AD, MEHTA RG, MOON RC, PEZZUTO JM (1997) Cancer chemopreventive activity of resveratrol, a natural product derived from grapes. Science 275: 218-220
34. JAYATILAKE GS, JAYASURIYA H, LEE ES, KOONCHANOK NM, GEAHLEN RL, ASHENDEL CL, MCLAUGHLIN JL, CHANG CJ (1993) Kinase inhibitors from *Polygonum cuspidatum*. J Nat Prod 56: 1805-1810
35. ZHANG CZ, WANG SX, ZHANG Y, CHEN JP, LIANG XM (2005) In vitro estrogenic activities of Chinese medicinal plants traditionally used for the management of menopausal symptoms. J Ethnopharmacol 98: 295-300