antioxidant capacity with a similar profile. Moreover, the ORAC assay demonstrated a clear enhancement of the antioxidant content in the *C. officinale* extract compared with the ascorbic acid, as standard.

The stable DPPH radical model is a widely used, relatively quick method for the evaluation of free radical scavenging activity. The effect of plant antioxidants on DPPH radical scavenging is thought to be due to their hydrogen donating ability.[33] The decrease in absorbance of DPPH radical caused by antioxidants because of the reaction between antioxidant molecules and radical, progresses, which results in the scavenging of the radical by hydrogen donation. Table 1 illustrates a significant decrease in the concentration of DPPH radicals due to the scavenging ability of the both *C. officinale* and *L. chuanxiong* extracts and standard. Free radical scavenging activity also increased with increasing concentration. These results indicated that both the extracts have a noticeable effect on scavenging free radicals. The methanol extract of *L. chuanxiong* showed a stronger DPPH scavenging activity than the *C. officinale* methanol extract when compared with standard. We used ascorbic acid as standard.

In addition, the ability to scavenge specific radicals may be targeted. Because different ROS have different reaction mechanisms, to completely determine antioxidant activity against a wide range of ROS, a more comprehensive set of assays needs to be carried out.[32] Superoxide anion ($O_2^{\bullet -}$) radical is an important factor in biological systems. In order to determine whether inhibition of NBT reduction was due to superoxide scavenger activity, a non-enzymatic system of superoxide generation was used. In the PMS-NADH-NBT system, superoxide anion, derived from dissolved oxygen from the coupling reaction of PMS-NADH, reduces NBT. The decrease in absorbance at 560 nm with antioxidants indicates the consumption of superoxide anion in the reaction mixture. Table 2 shows the percent inhibition of superoxide radical generation by 0–150 μg/ml of *C. officinale* and *L. chuanxiong* methanol extracts compared to that shown by ascorbic acid. *C. officinale* and *L. chuanxiong* methanol extracts showed a dose-dependent inhibition of superoxide radicals. Both the extracts of *C. officinale* and *L. chuanxiong* have strong superoxide radical scavenging activity (IC_{50} = 96.30 and 93.85 μg/ml). Considering the results obtained, it may be anticipated that the methanol extracts of *C. officinale* and *L. chuanxiong* have antioxidant activity, shown here by the scavenging of superoxide radical. IC_{50} values of all these extracts were greater than that of ascorbic acid in which IC_{50} was achieved at 8.76 μg concentration.

Scavenging of H_2O_2 by both the extracts may be attributed to their phenolics, which can donate electrons to H_2O_2, thus neutralizing it to water. The H_2O_2 scavenging capacities between the two extracts may be attributed to their electron donating abilities.[34] The ability of the both the extracts to effectively scavenge H_2O_2 is displayed in Table 2, in which it is compared with that of ascorbic acid as standard. The extracts were capable of scavenging H_2O_2 in a concentration-dependent manner. *C. officinale* and *L. chuanxiong* extracts (0–150 μg/ml) exhibited IC_{50} of 136.28 and 136.32 μg/ml, respectively, while ascorbic acid showed 8.05 μg/ml. The correlation between the *C. officinale* and *L. chuanxiong* values was statistically nonsignificant. Although H_2O_2 itself is not very reactive, it can sometimes cause cytotoxicity by giving rise to hydroxyl radicals in the cell. Thus, removing H_2O_2 is very important throughout food systems.

The *C. officinale* and *L. chuanxiong* methanol extracts were also evaluated for their ability to scavenge hydroxyl radicals using the deoxyribose degradation assay. In this study, the results showed that all samples were able to inhibit deoxyribose degradation (0–150 μg/ml), with a similar profile. The biochemical studies revealed that *C. officinale* and *L. chuanxiong* caused a concentration-dependent inhibition of deoxyribose degradation. At the IC_{50} value level, *C. officinale* (119.44 μg/ml) and *L. chuanxiong* (113.11 μg/ml) exhibited the same potency [Table 2]. Total OH radical scavenging capacities of each extract were compared to that of ascorbic acid.

Plant extracts were measured and compared for their free radical scavenging activities against nitric oxide radicals. The $NO^{\bullet}$ scavenging activity of *C. officinale* and *L. chuanxiong* methanol extracts was examined using SNP as a $NO^{\bullet}$ donor. NO released from SNP reacts with oxygen to produce

Table 2: Free radical scavenging and metal chelating activities (IC_{50} μg/ml) of methanol extracts of *C. officinale* and *L. chuanxiong*

	$O_2^{\bullet}$	H_2O_2	$OH^{\bullet}$	$NO^{\bullet}$	Metal chelation
CO	96.259 ± 8.024	136.280 ± 2.307	119.442 ± 7.444	57.252 ± 8.973	138.425 ± 13.292
LC	93.848 ± 9.529	136.318 ± 2.626	113.107 ± 8.890	76.502 ± 3.033[a*]	17.451 ± 5.858[a*]
AA[b]	8.762 ± 4.569	8.053 ± 3.677	3.034 ± 0.191	9.885 ± 0.478	43.235 ± 8.543

Values are means ± SD of three measurements. CO: *C. officinale*, LC: *L. chuanxiong*, AA: Ascorbic acid, $O_2^{\bullet -}$: superoxide radical, H_2O_2: hydrogen peroxide, $OH^{\bullet}$: hydroxyl radical, $NO^{\bullet}$: nitric oxide radical, [a]Different between CO and LC, *$P < 0.05$ (ANOVA/Tukey), [b]positive control

nitrite. NO scavenger competes with oxygen in reacting with NO• released from SNP solution in PBS. In this study, extracts from *C. officinale* and *L. chuanxiong* showed NO• scavenging capacity [Table 2], although some differences were noted. NO• scavenging activity of *C. officinale* was more significant than *L. chuanxiong*. This inhibition might also be a result of direct scavenging of NO• by extracts. *C. officinale* had the greatest activity to quench NO radical. The IC_{50} values were 57.25 and 76.50 μg/ml for *C. officinale* and *L. chuanxiong*, respectively.

The chelation of ferrous ions by *C. officinale* and *L. chuanxiong* extracts was estimated, in which ferrozine quantitatively forms complexes with Fe^{2+}. In the presence of chelating agents, the formation of this complex is disrupted, thereby impeding the formation of the red color imparted by the complex as well. Measurement of this color change therefore allows for the estimation of the chelating activity of the coexisting chelator.[35] In this assay, both the extracts and the standard antioxidant compound interfered with the formation of ferrous–ferrozine complex, suggesting that they have chelating activity, capturing the ferrous ion before it can form a complex with ferrozine. As shown in Table 2, the formation of the Fe^{2+}–ferrozine complex is not complete in the presence of the *C. officinale* and *L. chuanxiong* methanol extracts, indicating that both the extracts chelate the iron. The absorbance of Fe^{2+}-ferrozine complex linearly decreased in a dose-dependent manner (0–150 μg/ml). The difference between both the extracts of *C. officinale* and *L. chuanxiong*, and the control was statistically significant. The metal chelating capacities of methanol extracts of *C. officinale* and *L. chuanxiong*, and ascorbic acid (all at IC_{50} μg/ml) were 138.43, 17.45, and 43.24, respectively, which proved to be a significant difference between the extracts and the controls.

To determine the effects of *C. officinale* and *L. chuanxiong* on cell viability, the N2a cells were exposed to *C. officinale* and *L. chuanxiong* (50–500 μg/ml) for an incubation time of 1 h. In Figure 1, the MTT test after 1 h of incubation with C. officinale does not indicate any significant viability difference in treated N2a cell cultures in comparison with control. By MTT test after 1 h with *L. chuanxiong*, a significant increase of viability was observed in *L. chuanxiong* 500 μg/ml treated N2a cells in comparison with control. As shown in Figure 2, NO determination was performed after 1 h of incubation in the presence of *C. officinale* and *L. chuanxiong* (50–500 μg/ml). Treatment with *L. chuanxiong* did not decrease the release of NO significantly when compared to control, but 500 μg/ml *C. officinale* decreased significantly the NO release. From this result, it can be concluded that the methanolic extracts of *C. officinale* and *L. chuanxiong*, at the doses used, have no toxicity effects.

CONCLUSION

Free radical scavenging methods and modifications have been proposed to evaluate antioxidant characteristics and to explain how antioxidant molecules function. Of these, antioxidant activity, free radical scavenging and metal chelation are most commonly used for the evaluation of the total antioxidant behavior of extracts. In the present study, the various free radical scavenging activities of *C. officinale* and *L. chuanxiong* methanol extracts may be attributed to its strong abilities as a hydrogen donor. Moreover, the effectiveness of the methanol extracts of *C. officinale* and *L. chuanxiong* on the cell viability and nitric oxide release in cell culture model has also been established. Results of the present study showed that the selected species of the family Umbelliferae having potential in scavenging of free radicals come out for the therapeutic value.

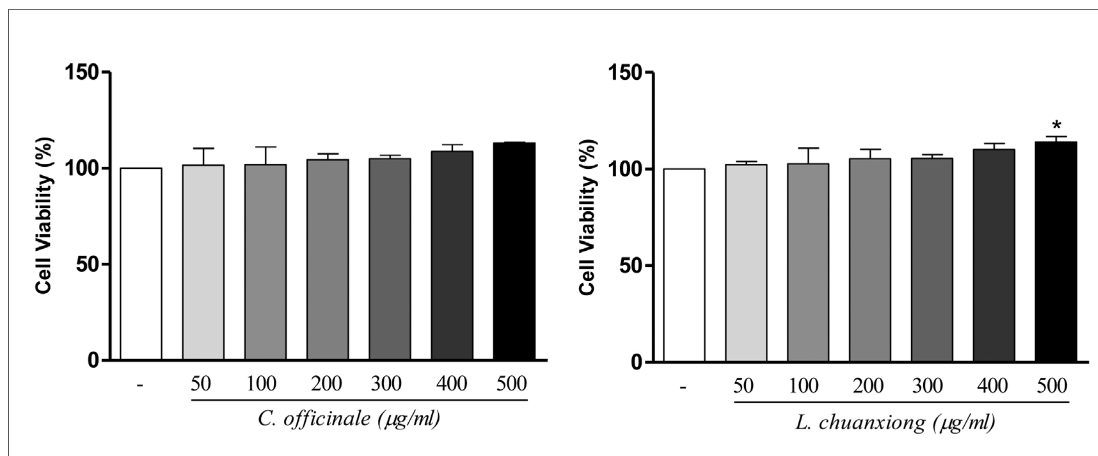

Figure 1: Effect of *C. officinale* and *L. chuanxiong* methanolic extracts on cell viability in N2a cells. Values are means ± SD of three measurements. *$P < 0.05$ compared with untreated normal (ANOVA/Tukey)

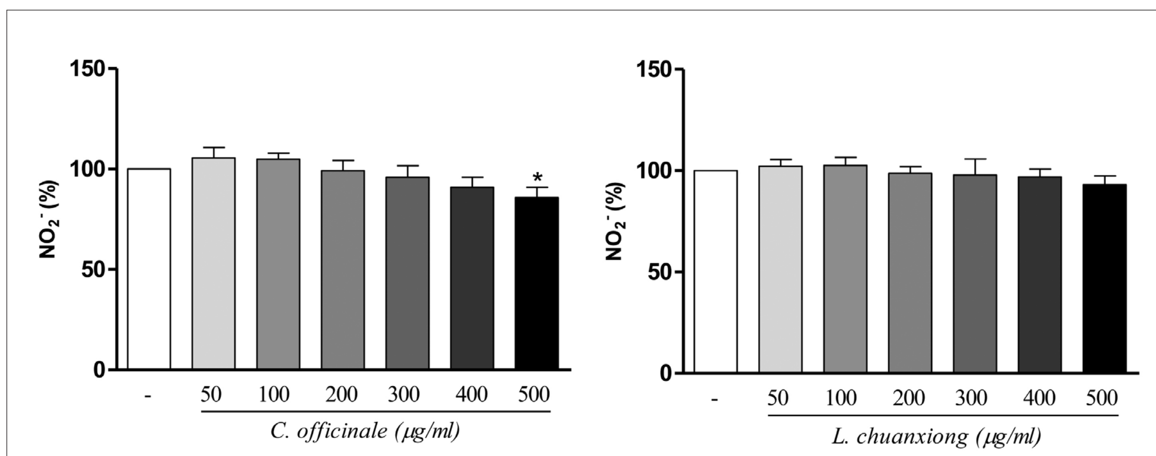

Figure 2: Effect of *C. officinale* and *L. chuanxiong* methanolic extracts on nitric oxide release in N2a cells. Values are means ± SD of three measurements. $^{*}P < 0.05$ compared with untreated normal. (ANOVA/Tukey)

REFERENCES

1. Moskovitz J, Yim KA, Choke PB. Free radicals and disease. Arch Biochem Biophys 2002;397:354-9.
2. Khanna D, Sethi G, Ahn KS, Pandey MK, Kunnumakkara AB, Sung B. Natural products as a gold mine for arthritis treatment. Curr Opin Pharmacol 2007;7:344-51.
3. Fusco D, Colloca G, Lo Monaco MR, Cesari M. Effects of antioxidant supplementation on the aging process. J Clin Interv Aging 2007;2:377-87.
4. Ahn MY, Ryu KS, Lee YW, Kim YS. Cytotoxicity, L-amino acid oxidase activity of crude insect drugs. Arch Pharm Res 2000;23:477-81.
5. Jeong SI, Kwak DH, Lee S, Choo YK, Woo WH, Keum KS, *et al*. Inhibitory effects of *Cnidium offcinale* Makino and *Tabanus fulvus* Meigan on the high glucose-induced proliferation of glomerular mesangial cells. Phytomed 2005;12:648-55.
6. Li SL, Yan R, Tam YK, Lin G. Post-harvest alteration of the main chemical ingredients in *Ligusticum chuanxiong* Hort. (Rhizoma Chuanxiong). Chem Pharm Bull 2008;55:140-4.
7. Yuen L. Herbal skin regeneration composition and method. US patent 2000;6,027:728.
8. Meepagala K, Sturtz G, Wedgem CE, Schraderm KK, Duke SO. Phytotoxic and antifungal compounds from two apiaceae species, *Lomatium clifornicum* and *Ligusicum hultenii*, rich sources of z-ligustilide and apiol, respectively. J Chem Ecol 2005;31:1567-78.
9. Tsukamoto T, Ishkawa U, Miyazawa M. Larvicidal and adulticidal activity of alkylphthalide derivatives from rhizome of *Cnidium officinale* against *Drosophila melanogaster*. J Agric Food Chem 2005;53:5549-53.
10. Beck JJ, Chou SC. The Structural diversity of phthalides from the Apiaceae. J Nat Prod 2007;70:891-900.
11. Zhang C, Qi M, Shao Q, Zhou S, Fu R. Analysis of the volatile compounds in *Ligusticum chuanxiong* Hort. using HS-SPME-GC-MS. J Pharmaceut Biomed Anal 2007;44:464-70.
12. Tahara E, Satoh T, Toriizuka K, Nagai H, Nunome S, Shimada Y, *et al*. Effect of Shimotsu-to (a kampo medicine, Si-Wu-Tang) and its constituents on triphasic skin reaction in passively sensitized mice. J Ethnopharmacol 1999;68:219-28.
13. Chung MS. Volatile Compounds of *Cnidium officinale* MAKINO. Food Sci Biotechnol 2004;13:603-8.
14. Jeong JB, Ju SY, Park JH, Lee JR, Yun KW, Kwon ST, *et al*. Antioxidant activity in essential oils of *Cnidium officinale* makino and *Ligusticum chuanxiong* hort and their inhibitory effects on DNA damage and apoptosis induced by ultraviolet B in mammalian cell. Cancer Epidemiol 2009;33:41-6.
15. Zhu S, Fushimi H, Han G, Tsuchida T, Uno T, Takano A, *et al*. Molecular identification of "Chuanxiong" by nucleotide sequence and multiplex single base extension analysis on chloroplast *trn*K gene. Biol Pharm Bull 2007;30:527-31.
16. Lee SH, Choi HW, Sung JS, Bang JW. Interspecific relationships among three medicinal species: *Cnidium officinale*, *Lingustcum chuanxiong* and *Angelica polymorpha*. Plant and Animal Genomes XVII conference; 2009 Jan 10-14; USA: San Diego.
17. Park YK. The study on antioxidative effects and quality comparison of *Ligusticum chuanxiong* and *Cnidium officinale* (1). Korean J Herbol 1998;13:103-8.
18. Lee HW, Cho HG, Park YK. The study on antioxidative effects and quality comparison of *Ligusticum chuanxiong* and *Cnidium officinale* (2). Vascular relaxant effect of Cnidii rhizome and Cnidii rhizome-Angelicae radix compound. Korean J Herbol 1999;14:55-60.
19. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improvement ABTS radical cation decolourization assay. Free Radic Biol Med 1999;26:1232-7.
20. Schlesier K, Harwat M, Böhm V, Bitsch R. Assessment of antioxidant activity by using different *in vitro* methods. Free Radic Res 2002;36:177-87.
21. Cao G, Verdon CP, Wu AH, Wang H, Prior RL. Automated assay of oxygen radical absorbance capacity with the COBAS FARA 2. Clin Chem 1995;41:1738-44.
22. Gyamfi MA, Yonamine M, Aniya Y. Free-radical scavenging action of medicinal herbs from Ghana: *Thonningia sanguinea* on experimentally induced liver injuries. Gen Pharmacol 1999;32:661-7.
23. Robak J, Gryglewski RJ. Flavonoids are scavengers of superoxide anions. Biochem Pharmacol 1988;36:317-22.
24. Sinha AK. Colorimetric assay of catalase. Anal Biochem 1972;47:389-94.
25. Ohkawa H, Ohishi N, Yagi K. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal Biochem 1979;95:351-8.
26. Chung SK, Osawa T, Kawakishi S. Hydroxyl radical scavenging effects of spices and scavengers from Brown Mustard (*Brassica*

nigra). Biosci Biotech Biochem 1997;61:118-23.

27. Garrat DC. The quantitative analysis of drugs. Vol. 3. Japan: Chapman and Hall; 1964.
28. Marcocci PL, Sckaki A, Albert GM. Antioxidant action of *Ginkgo biloba* extracts EGP761. Methods Enzymol 1994;234:462-75.
29. Decker EA, Welch B. Role of ferritin as a lipid oxidation catalyst in muscle food. J Agric Food Chem 1990;38:674-7.
30. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 1982;126:131-8.
31. Rice-Evans CE, Miller NJ. Total antioxidant status in plasma and body fluids. Meth Enzymol 1994;234:279-83.
32. Wang H, Cao G, Prior R. Total antioxidant capacity of fruits. J Agric Food Chem 1996;44:701-5.
33. Baumann J, Wurn G, Bruchlausen FV. Prostaglandin synthetase inhibiting O_2-radical scavenging properties of some flavonoids and related phenolic compounds. Naunyn-Schmiedebergs Arch Pharmacol 1979;308:27-32.
34. Wettasinghe M, Shahidi F. Scavenging of reactive-oxygen species and DPPH free radicals by extracts of borage and evening primrose meals. Food Chem 2000;70:17-26.
35. Yamaguchi F, Ariga T, Yoshimira Y, Nakazawa H. Antioxidative and anti-glycation activity of garcinol from *Garcinia indica* fruit rind. J Agric Food Chem 2000;48:80-5.

Source of Support: Oriental Medicine R&D Project, Ministry of Health, Welfare & Family Affairs, Republic of Korea (B080027 & B090075), **Conflict of Interest:** None declared