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# The efficiency of flavonoids in polar extracts of *Lycium chinense* Mill fruits as free radical scavenger

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**Abstract**

The extracts of *Lycium chinense* Mill fruits with water, 50% ethanol and 95% ethanol were compared in antiradical efficiency according to the Chinese consumption tradition. The results showed that water infusion was similar to 50% ethanol extract in flavonoids ($1154 \pm 89$ and $1207 \pm 66$ mg $kg^{-1}$, respectively), 95% ethanol extract contained more flavonoids ($1497 \pm 70$ mg $kg^{-1}$). The main flavonoids in *Lycium chinense* Mill fruits were rutin and chlorogenic acid in water extract, rutin and protocatechuic acid in 50% ethanol extract and rutin in 95% ethanol extract as determined with high-performance liquid chromatography (HPLC). All extracts showed a slow kinetics in DPPH· antiradical reaction system. The antiradical efficiency of the extracts tended to become stronger as the polarity of the solvents decreased.


*Keywords:* Boxthorn (*Lycium chinense* Mill); Antiradical efficiency; DPPH·

## 1. Introduction

Originated from China and now widely planted in warm and subtropical countries such as Japan, Korea and other southeastern Asian and European countries (Hou, 1984), boxthorn (*Lycium chinense* Mill) is regarded as an important and a good foodstuff, since approximate analysis shows that there are 55.4 g $kg^{-1}$ of protein, 10.4 g $kg^{-1}$ of fat, 267.3 g $kg^{-1}$ of carbohydrates, 37.4 g $kg^{-1}$ of crude fibre, 13.8 g $kg^{-1}$ of minerals, 761 mg $kg^{-1}$ of calcium, 1191 mg $kg^{-1}$ of phosphorous, 19 mg $kg^{-1}$ of iron, 119 mg $kg^{-1}$ of carotene, 7 mg $kg^{-1}$ of thiamine, 11 mg $kg^{-1}$ of riboflavin, 26 $kg^{-1}$ of nicotinic acid and 470 mg $kg^{-1}$ of ascorbic acid in dry fruits (CAMS, 1980). In China, the fruits are infused with water or spirit liquor and cooked with broth of poultry or domestic animal meat, as boxthorn is a good visual acuity improver according to the traditional Chinese medicine (mechanism unknown). Of the cultivars approximately numbered 80, the one popularly cultivated in Ningxia Hui Autonomous Region, China, is the most famous.

Researchers have laid their interests in elucidating the functional chemical composition in boxthorn and their functions, those who are in China have devoted their efforts on polysaccharides and have published hundreds of theses, and those who are outside China have been engaged in other functional constituents including antioxidants, polysaccharides, alkaloids, glycopeptides, glycoprotein, peptides and tocopherols, etc. (Chin et al., 2003; Ching & Mohamed, 2001; Funayama, Yoshida, Konno, & Hikino, 1980; Funayama, Zhang, & Nozoe, 1995; Geng, Xin, & Zhou, 1988; Hsu, Yang, Ho, & Lin, 1999; Khan, Qayum, & Qureshi, 1969; Kim, Kim, & Kim, 1998; Lam & But, 1999; Miana, 1973; Oishi, Mochizuki, Takasu, & Nakamura, 1996; Qin, Kato, Yamauchi, Aizawa, & Inakuma, 1999; Qin, Yamauchi, Aizawa, Inakuma, & Kato, 2000; Qin, Yamauchi, Aizawa, Inakuma, & Kato, 2001; Sannai, Fujimori, & Kat, 1980; Terauchi, Kanamori, Nobuso, Fukuda, Yahara, & Yamasaki, 1998; Tian & Wang, 1995; Yahara, Shigeyama, Nohara, Okuda, Wakamatsu, & Yasuhara, 1989; Yoon, Jeon, Oh, Lee, & Jeong, 2000; Yoon, Kim, Chae, Oh, & Lee, 2001).

Conventionally, the Chinese use to consume the infusion of boxthorn fruit with water and spirit liquor

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with an ethanol concentration ranging from 45% to 60% (v/v). The present work aimed at simulating the normal consumption method with polar solvents and finding the flavonoids and their functions in boxthorn fruits.

## 2. Materials and methods

### 2.1. Materials

The fruits of boxthorn cultivated in Ningxia Hui Autonomous Region, People's Republic of China, were purchased from a local supermarket. The reference chemical reagents were purchased from authentic producers, hyperoside from Extrasytheses (Gemay, France), rutin, hesperidin, morin, chlorogenic acid, 2-aminoethyl-diphenylborate, and 1,1-diphenyl-2-picryl-hydrazyl (DPPH·) from Sigma–Aldrich (Steinheim, Germany) and protocatechuic acid and quercetin from Fluka (Buchs, Switzerland). All the chemicals used were of guaranteed grade.

### 2.2. Preparation and extraction

The boxthorn fruits were dried at 105 °C for 4 h in an oven, followed by being ground to the size of about 1 mm with a lab mill. 10.00 g of meal samples were weighed into 100 ml volumetric flasks, and repeatedly distilled water, 50% ethanol aqueous solution and 95% ethanol were added to the mark, respectively. The flasks were cap locked and put into water bath at 90 °C to extract for 2 h. After the suspensions were filtered with a 200-mesh sieve, the filtrates were adjusted to 100 ml with virgin solvents and ready for consequent analyses.

### 2.3. Measurement of total flavonoids

The contents of total flavonoids were quantitatively assayed assuming a spectrophotometric procedure discribed by Hariri, Sallé, and Andary (1991) with rutin as reference in calibration instead of quercetol. The filtered extracts were not treated furthermore, except necessary diluting operations. 1% methanol solution of 2-aminoethyl-diphenylborate served as colour developer. The density of opaque was detected at the wavelength of 404 nm using a UV-2401 PC UV–Vis Recording Spectrophotometer (Shimadzu, Japan). Three to four measurements were replicated for each sample to obtain the average values and standard deviations (mean ± SD).

### 2.4. Analyses of flavonoids with high-performance liquid chromatography

The high-performance liquid chromatography (HPLC) system consisted of the following units. Instrument: Waters 600/2487 HPLC (Milford, MA, USA); M32 work station software; column: Diamonsil $C_{18}$, ∅ 4.6 mm × 25 cm. Operating conditions were detecting wavelength: 258 nm; column temperature: 30 °C; mobile phase: solvent A, acetonitrile/water/acetic acid (5/94.5/0.5, v/v/v); solvent B, acetonitrile/water/acetic acid (70/29.5/0.5, v/v/v). flow rate: 1 ml $min^{-1}$; gradient conditions: 0–25 min 100–80% A, 0–20% B; 25–35 min 80–65% A, 20–35% B; 35–55 min 65–20% A, 35–80% B; 50–55 min 20–0% A, 80–100% B; 55–65 min 100% A. Injection volume: 10 μl.

In order to protect the chromatographic column, the water extract was treated with absolute ethanol in the same volume to precipitate possibly solved proteins and carbohydrates and all the extracts were filtered with a micro-filter of 125 μm in diameter before injection. A mixture of hesperidin, morin, chlorogenic acid, hyperoside, rutin, quercetin and protocatechuic acid was used as standard.

### 2.5. Effect of extracts to quench free radical

The three extracts of boxthorn, water, 50% ethanol and 95% ethanol, were applied to a DPPH· free radical reaction system to determine the scavenging activity and kinetics (Brand-Williams, Cuvellier, & Berset, 1995). In this method, the exact initial DPPH· concentration ($C_{DPPH}$) in the reaction medium was calculated from a calibration curve with the equation, $Abs_{515\,nm} = 0.7784 \times (C_{DPPH}) - 5.3 \times 10^{-3}$, as determined by linear regression.

For each extract with deferent flavonoids concentration tested, the reaction kinetics was plotted. From these

Table 1
Composition and content of flavonoids in extracts (mg $ml^{-1}$)

| | Water extract | 50% Ethanol extract | 95% Ethanol extract | Peak in Fig. 1 |
|---|---|---|---|---|
| Protocatechuic acid | 0.21 | 1.8 | 0.17 | A |
| Chlorogenic acid | 1.01 | 0.17 | 0.23 | B |
| Rutin | 0.86 | 2.79 | 3.40 | C |
| Hyperoside | 0.032 | 0.021 | 0.13 | D |
| Hesperidin | tr* | tr* | tr* | |
| Morin | 0.049 | tr* | 0.047 | E |
| Quercetin | 0.25 | 0.089 | 0.16 | F |

Data obtained from HPLC analyses.
* <0.01 mg $ml^{-1}$.

graphs, the percentage of DPPH· remaining at the steady state was determined and the values transferred onto another graph showing the percentage of residual DPPH· at the steady state as a function of the molar ratio of flavonoids (expressed in the form of rutin as representative) in extracts to DPPH·. Free radical scavenging activity was defined as the amount of flavonoids necessary to decrease the initial DPPH·

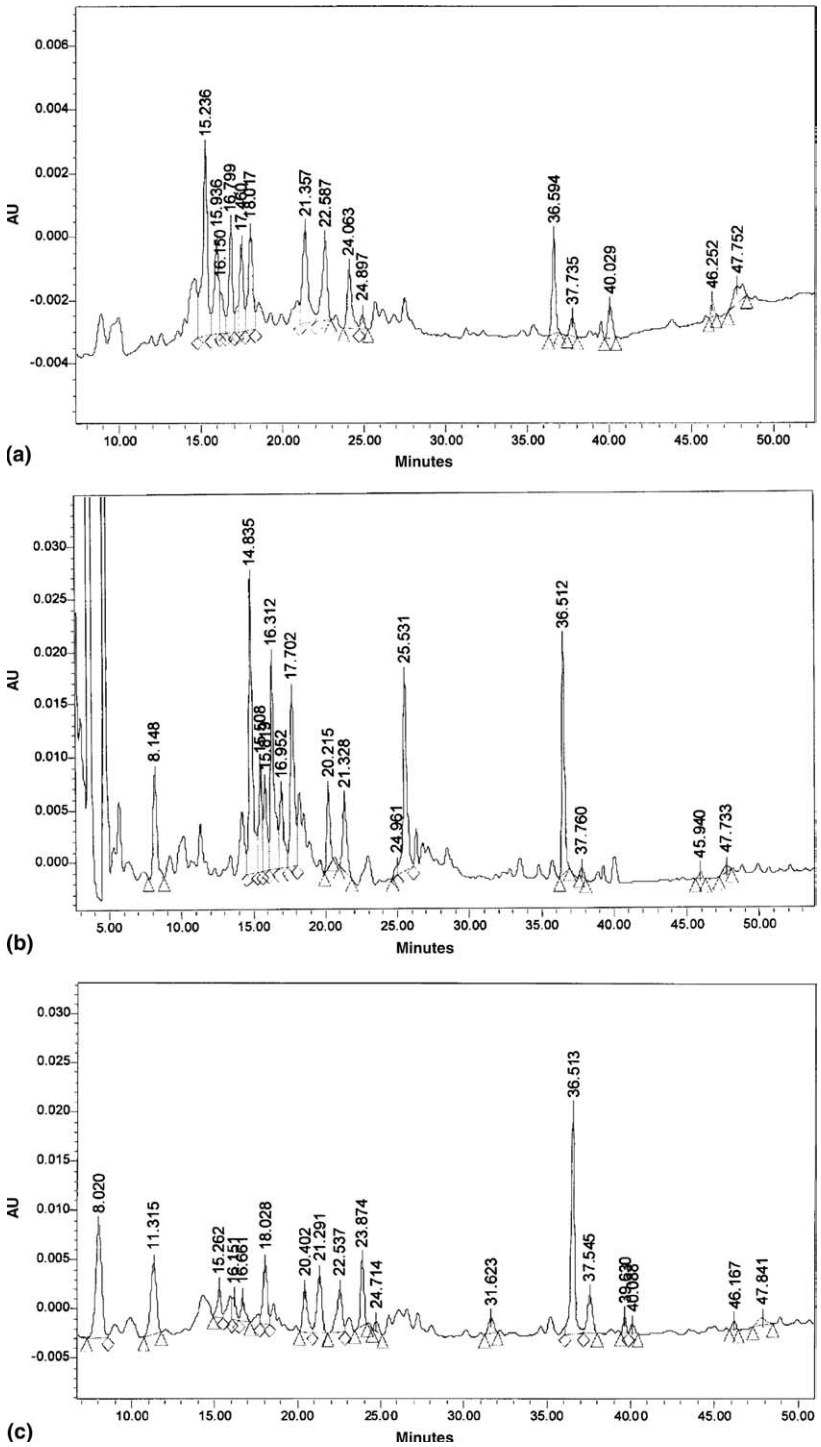


Fig. 1. High-performance chromatograms of: (a) water extract; (b) 50% ethanol extract and (c) 95% ethanol extract. The migration time for protocatechuic acid (referred as Peak A) is 16.150 min in (a), 16.312 min in (b) and 16.151 min in (c); chlorogenic acid (Peak B), 24.063 min (a), 24.961 min (b) and 24.714 min (c) min; rutin (Peak C), 36.594 min (a), 35.512 min (b) and 36.513 min (c); hyperoside (Peak D), 37.735 min (a), 37.760 min (b) and 37.545 min (c); morin (Peak E), 46.252 min (a), and 46.167 min (c) and quercetin (Peak F), 47.733 min (a), 47.733 min (b) and 47.841 min (c), respectively.

concentration by 50% ($EC_{50}$). The $1/EC_{50}$ was referred to the antiradical power (ARP), obviously, the larger the ARP, the more efficient the extract.

## 3. Results and discussion

### 3.1. Total flavonoids in L. chinense fruist

The flavonoids content in water extract, 50% ethanol extract and 95% ethanol extract are $1154 \pm 89$, $1207 \pm 66$ and $1497 \pm 70$ mg $kg^{-1}$, respectively. The amounts are up to 10 times those in fine buckwheat flour (140 mg $kg^{-1}$) (Qian, Mayer, & Kuhn, 1999), which is a good source of flavonoids. Surely, boxthorn is a good flavonoid supply, too. According to the method of Hariri et al. (1991), the total flavonoids in fruits should be 1522 mg $kg^{-1}$, consequently, 75.82%, 79.30% and 98.36% of which could be extracted in the sequence of solvents from water to 95% ethanol, respectively.

### 3.2. Flavonoids composition as assayed with HPLC

There seems no common rules can be established from the data in Table 1 and Fig. 1. Although the polarity decreases in the sequences of water, 50% ethanol and 95% ethanol, the solutes, flavonoids, in different extracts do not show any relationship with the property of solvents. For example, no evidence in the result supports that rutin has the same soluable behavior as hyperoside does, although they have the same number of functional groups. Protocatechuic acid (1.8 mg $ml^{-1}$) and rutin (2.79 mg $ml^{-1}$) are two major components in 50% ethanol extract, chlorogenic acid in water extract predominates (1.01 mg $ml^{-1}$), while in 95% ethanol extract the content of rutin (3.40 mg $ml^{-1}$) is so high. Hesperitin appears in the three extracts in trace amount. Sannai et al. (1980) reported (−)-1,2-dehydro-cyperone and solavetivone in boxthorn. However, as limited by the number of standard samples, all the constituents are not identified in the present work.

### 3.3. Efficiency of extracts to quench free radical

DPPH· is a stable free radical, and researchers have been using this reaction system to evaluate the efficiency of antioxidants and flavonoids (Bondet, Brand-Williams, & Berset, 1997; Brand-Williams et al., 1995; Sánchez-Moreno, Larrauri, & Saura-Calixto, 1998). These extracts are heterogeneous in components (Fig. 2), therefore, it is difficult to describe the reaction kinetics. Nevertheless, the investigating system drops into the slow kinetics as classified by Brand-Williams et al. (1995), since it takes 4–5 h to reach steady state as the amount of extract in the reaction system differs. The steady state was used to determine the $EC_{50}$ as Sánchez-Moreno et al. (1998) had pointed out that reaction time affected the antiradical efficiency, especially in slow kinetics. There existed a somewhat difference between the two classifications. For example, Brand-Williams et al. (1995) considered gallic acid slow kinetics behavior, while Sánchez-Moreno et al. (1998) classified it into medium. The $EC_{50}$ for water extract is 0.26 mol flavonoids/mol DPPH· (ARP 3.85), 0.21 mol flavonoids/mol DPPH· (ARP 4.76) for 50% ethanol extract and 0.20 mol flavonoids/mol DPPH· (ARP 5.0) for 95% ethanol extract (Fig. 3). Compared with pure chemicals in the same system, the extracts show better antiradical power than commonly used antioxidants such as ascorbic acid (ARP 3.70), δ-tocopherol (ARP 4), butylated hydroxyanisole (ARP 4.17) and butylated hydroxytoluene (ARP 4.20) (Brand-Williams et al., 1995). High concentration is beneficial to the ARP of extract.

Briefly in conclusion, the infusion of *L. chinense* Mill fruits with water is similar to that with 50% ethanol, and 95% ethanol can extract more flavonoids and is stronger in free radical scavenging. Polar solvents can extract most of the flavonoids in *L. chinense* fruits. It suggests

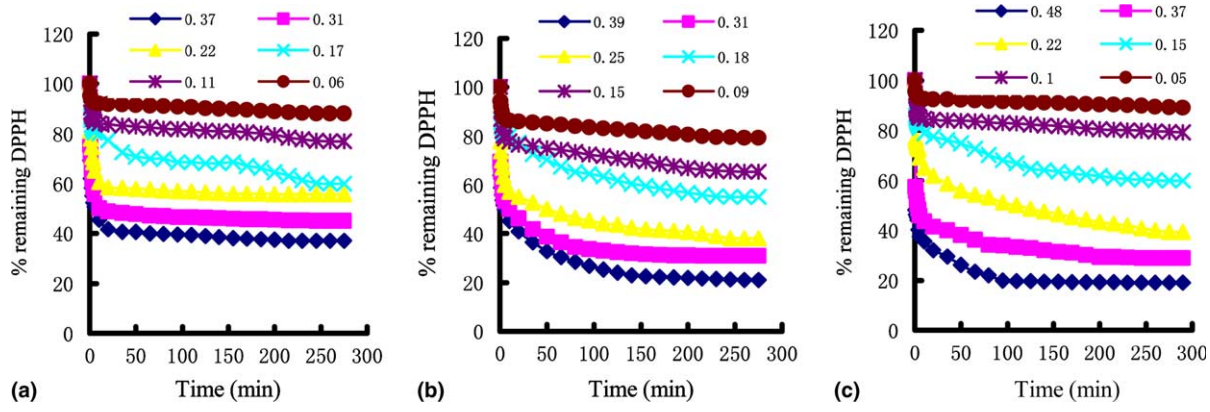


Fig. 2. Reaction kinetics of extract to quench free radical (a) water extract; (b) 50% ethanol extract and (c) 95% ethanol extract. Number in legends means the concentration of flavonoids in extract (mg $ml^{-1}$).

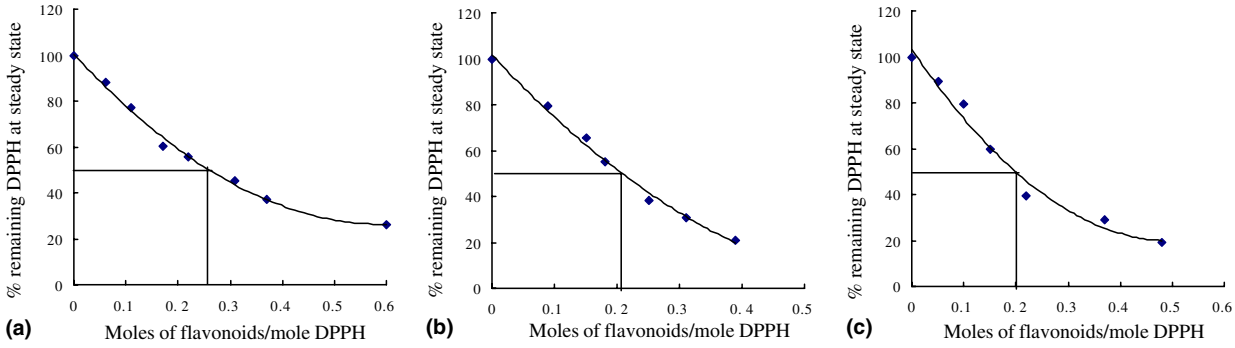


Fig. 3. The disappearance of DPPH· as a function of the number of moles of flavonoids/mole DPPH·, where the arrow points to is $EC_{50}$. (a) Water extract; (b), 50% ethanol extract and (c) 95% ethanol extract.

that the Chinese people consume *L. chinense* fruits in an effective way.

Academically, further work could be carried out with apolar solvents, with which seldom work has been reported to extract flavonoids, although.

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## References

Bondet, V., Brand-Williams, W., & Berset, C. (1997). Kinetics and mechanisms of antioxidant using the DPPH· free radical method. *Lebensmittel-Wissenschaft Und-Technologie, 30*, 609–615.

Brand-Williams, W., Cuvellier, M. E., & Berset, C. (1995). Use of a free radical to evaluate antioxidant activity. *Lebensmittel-Wissenschaft Und-Technologie, 28*, 25–30.

CAMS (1980). *Data book of food ingredients*. Beijing: People's Hygiene Publishing House, p. 85.

Chin, Y.-W., Lim, S. W., Kim, S.-H., Shin, D.-Y., Suh, Y.-G., Kim, Y.-B., Kim, Y. C., & Kim, J. W. (2003). Hepatoprotective pyrrole derivatives of *Lycium chinense* fruits. *Bioorganic & Mediclinal Chemistry Letters, 13*(1), 79–81.

Ching, L. S., & Mohamed, S. (2001). Alpha-tocopherol content in 62 edible tropical plants. *Journal of Agricultural Food Chemistry, 49*(6), 3101–3105.

Funayama, S., Yoshida, K., Konno, C., & Hikino, H. (1980). Structure of kukoamine A, a hypotensive principle of *Lycium chinense* root barks1. *Tetrahedron Letters, 21*(14), 1355–1356.

Funayama, S., Zhang, G.-R., & Nozoe, S. (1995). Kukoamine B, a spermine alkaloid from *Lycium chinense*. *Phytochemistry, 38*(6), 1529–1531.

Hariri, E. B., Sallé, G., & Andary, C. (1991). Involvement of flavonoids in the resistance of two polarcultivars to mistletoe (*Viscum album* L.). *Protoplasma, 162*, 20–26.

Geng, C., Xin, S., & Zhou, J. (1988). Immunomudulating action of *Lycium baebarum* polysaccharids. *Intrenational Immunopharmacology, 10*(S1), 50.

Hou, K. (1984). *A dictionary of the families and genera of Chinese seed plants* (2nd ed.). Beijing: Science Press, p. 286.

Hsu, H.-Y., Yang, J.-J., Ho, Y.-H., & Lin, C.-C. (1999). Difference in the effects of radioprotection between aerial and root parts of *Lycium chinense*. *Journal of Ethnopharmacology, 64*(2), 101–108.

Khan, I., Qayum, A., & Qureshi, Z. (1969). Study of the hypotensive action of berbamine, an alkaloid isolated from berberis lycium. *Life Science Part 1, 8*(17), 993–1001.

Kim, S. K., Kim, Y. C., & Kim, Y. C. (1998). Effects of singly administered betaine on hepatotoxicity of chloroform in mice. *Food Chemical Toxicology, 36*(8), 655–661.

Lam, K. W., & But, P. (1999). The content of zeaxanthin in gou qi zi, a potential health benefit to improve visual acuity. *Food Chemistry, 67*(2), 173–176.

Miana, G. A. (1973). Tertiary dihydroprotoberberine alkaloids of *Berberis lycium*. *Phytochemistry, 12*(7), 1822–1823.

Oishi, M., Mochizuki, Y., Takasu, T., & Nakamura, S. (1996). Effects of a traditional Chinese medicine on dementia. *Electroencephalography and Clinical Neurophysiology, 98*(4), 51.

Qian, J.-Y., Mayer, D., & Kuhn, M. (1999). Flavonoids in fine buckwheat (*Fagopyrum esculentum* Moench) flour and their free radical scavenging activities. *Deutsche Lebensmittel-Rundschau, 95*, 343–349.

Qin, X., Kato, K., Yamauchi, R., Aizawa, K., & Inakuma, T. (1999). Chemical features of water-soluble polysaccharides in *Lycium chinense* Mill fruit. *Research Bulletin Faculty Agriculture Gifu University, 64*, 83–88.

Qin, X., Yamauchi, R., Aizawa, K., Inakuma, T., & Kato, K. (2000). Isolation and characterization of arabinogalactan-protein from the fruit of *Lycium chinense* Mill. *Journal of Appllied Glycoscience, 47*(2), 155–161.

Qin, X., Yamauchi, R., Aizawa, K., Inakuma, T., & Kato, K. (2001). Structural features of arabinogalactan-proteins from the fruit of *Lycium chinense* Mill. *Carbohydrate Research, 333*(1), 79–85.

Sánchez-Moreno, C., Larrauri, J. A., & Saura-Calixto, F. (1998). A procedure to measure the antiradical efficiency of polyphenols. *Journal of the Science of Food and Agriculture, 76*, 270–276.

Sannai, A., Fujimori, T., & Kat, K. (1980). Isolation of (−)-1,2-dehydro-cyperone and solavetivone from *Lycium chinense*. *Phytochemistry, 21*(12), 2986–2987.

Terauchi, M., Kanamori, H., Nobuso, M., Fukuda, S., Yahara, S., & Yamasaki, K. (1998). Antimicrobial components in leaves of *Lycium chinense* Mill. *Journal of Food Hygienic Society Japan (Shokuhin-Eiseigaku-Zasshi), 39*(6), 399–405.

Tian, G.-Y., & Wang, C. (1995). Structure elucidation of a high MW glycan of a glycoprotein isolated from the fruit of *Lycium barbarum* L. *Acta Biochemiica Biophysinca Sinica, 27*(2), 201–205.

Yahara, S., Shigeyama, C., Nohara, T., Okuda, H., Wakamatsu, K., & Yasuhara, T. (1989). Structures of anti-ace and -renin peptides from lycii radicis cortex. *Tetrahedron Letters, 30*(44), 6041–6042.

Yoon, C. G., Jeon, T. W., Oh, M. J., Lee, G. H., & Jeong, J. H. (2000). Effect of the ethanol extract of *Lycium chinense* on the oxygen free radical and alcohol metabolizing enzyme activities in rats. *Journal of the Korean Society Food Science Nutrition, 29*(2), 268–273.

Yoon, C. G., Kim, H. H., Chae, S. N., Oh, M. J., & Lee, G. H. (2001). Hepatic oxygen free radical and alcohol metabolizing enzyme activities in rats fed diets supplemented with *Lycium chinense* ethanol extract. *Journal of the Korean Society Food Science Nutrition, 30*(4), 668–672.