Journal of Medicinal Plants Research Vol. 5(16), pp. 3702-3707, 18 August, 2011
Available online at http://www.academicjournals.org/JMPR
ISSN 1996-0875 ©2011 Academic Journals

*Full Length Research Paper*

# Antioxidant activities of berberine hydrochloride

**Aoxue Luo and Yijun Fan***

Department of Landscape Plants, Sichuan Agriculture University, Chengdu 611130, P. R. China.

Accepted 21 April, 2011

**In order to explore the mechanism of berberine hydrochloride in treating diabetes, antioxidant activities of the berberine hydrochloride *in vitro* were carried out. In the present study, our study aimed to examine the antioxidant activity of berberine hydrochloride using different assays including: reducing power, 2,2-diphenyl 1-picrylhydrazyl (DPPH) radical scavenging assay, ABTS radical scavenging assay, superoxide anion and hydroxyl radical scavenging activity. The results exhibited that berberine hydrochloride has significant reductive ability and radicals scavenging effects, especially on ABTS, hydroxyl radicals and DPPH radicals.**

**Key words:** Berberine hydrochloride, antioxidant activity, free radicals, *in vitro.*

## INTRODUCTION

Berberine hydrochloride, is an isoquinolin alkaloid from a number of important medicinal plant species such as the Berberidaceae, Ranunculaceae families. Many reports and studies have been shown that Berberine hydrochloride has various effects including anti-inflammatory (Zhou and Mineshita, 2000), anti microbial (Iwasa et al., 1998), anti-tumor (Fukuda et al., 1999), antipyretic (Esra et al., 2002). Recently, it was found that berberine hydrochloride was effective and safe in the treatment of diabetic patients (Yin et al., 2008). Diabetes is a serious, complex metabolic disorder and prevalent systemic disease affecting a significant proportion of the population worldwide. It is characterized by absolute or relative deficiencies in insulin secretion and/or insulin action associated with chronic hyperglycemia and disturbances of carbohydrate, lipid, and protein metabolism (Tang et al., 2006).

It is also known that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential (Tatiya et al., 2010). In diabetes mellitus several features appear including an increase in lipid peroxidation, alteration of the glutathione redox state, a decrease in the content of individual natural antioxidants, and finally a reduction in induction of antioxidant enzymes. And various studies have been confirmed that berberine hydrochloride could be effective in treatment of diabetes on diabetic rats due to its antioxidant properties (Zhou et al., 2009). Berberine hydrochloride significantly inhibited the progression of diabetes induced by alloxan, and the inhibitory effect of berberine hydrochloride on diabetes might be associated with its hypoglycemic effect, modulating lipids metabolic effects and its ability to scavenge free radical. So, how to scavenge free radicals and which free radicals could be scavenged by berberine hydrochloride on diabetes is very important. However, until now, there are almost no studies analyzing the free radical scavenging of berberine hydrochloride *in vitro*. Therefore, in order to understand the mechanisms of berberine hydrochloride in treating diabetes, the study was to elucidate antioxidant activities of the berberine hydrochloride *in vitro*.

*Corresponding autho. E-mail: Yijfan@yahoo.com.cn

## MATERIALS AND METHODS

### Drugs and reagents

BTH and Vitamin C were purchased from Sigma Chemical Co. 1,1-diphenyl-2-picryl-hydrazyl (DPPH), potassium ferricyanide [$K_3Fe(CN)_6$], trichloroacetic acid (TCA), polyoxyethylenesorbitan monolaurate (Tween-20), and berberine hydrochloride were purchased from Sigma (Sigma- Aldrich GmbH, Sternheim, Germany). Thiobarbituric acid (TBA), sodium dodecyl sulphate, nitroblue tetrazolium (NBT), nicotinamide adenine dinucleotide (NADH), and phenazine methosulphate (PMS) were purchased from Applichem. ABTS radical was purchased from Merck and all other chemicals were analytical grade and were made in China.

### Reducing power

The reducing power of berberine hydrochloride was quantified by the method described earlier by Raza et al. (2007) and Yen et al.

(1995) with some modifications. Vitamin C and BHT were used as reference material. Briefly, berberine hydrochloride, Vc and BHT were used at differing concentrations (1 to 4000 µg/ml). 1 ml of sample was mixed with phosphate buffer (2.5 ml, 0.2 mol/l, pH 6.6) and potassium ferricyanide [$K_3Fe(CN)_6$] (2.5 ml, 1%). The mixture was incubated at 50°C for 20 min. Then the reaction was terminated by 2.5 ml TCA solution (0.1%) and the mixture was centrifuged at 3000 rpm for 10 min. The supernatant (2.5 ml) was mixed with distilled water (2.5 ml) and ferric chloride (0.5 ml, 6 mmoll/l), and the absorbance was measured at 700 nm. Increased absorbance of the reaction mixture indicated increased reducing power.

#### Superoxide anion scavenging assay

Measurement of superoxide anion scavenging activity of berberine hydrochloride was based on the method described by Zhao et al. (2003) and Wang et al. (2008), with slight modification. 4.5 ml Tris–HCl buffer (50 mmol/L,pH 8.2) and 1.0 ml tested samples with various concentrations (1, 3, 10, 30, 100, 300, 1000, 2000, 3000 and 4000 µg/ml) were mixed in tubes with lids. Then the mixture was incubated for 20 min in the water bath at 25°C. Meanwhile, 0.4 ml of 25 mmol/L pyrogallol preheated at 25°C was added immediately. After 4 min, the reaction was terminated by 0.1 ml HCl solution (8 mol/L) and the mixture was centrifuged at 4000 rpm for 15 min. The absorbance of sample and control were determined by UV spectrophotometer at 325 nm. The curve was made based on the absorbance value. Vc was used as the positive control compounds. Scavenging activity was calculated using the following equation:

Superoxide anion scavenging effect (%) = $(A_0-A_s)/A_0 \times 100$

where $A_0$ is the absorbance without sample, and As is absorbance with sample.

#### ABTS radicals scavenging assay

The antioxidant activity of berberine hydrochloride was determined by ABTS radical cation as described by Fan et al. (2009) and Re et al. (1999), with some modifications. ABTS was dissolved in PBS (0.01 M, pH 7.4) to a 7 mM concentration. ABTS radical cation was produced by reacting reacting 7 mM ABTS solution with 2.45 mM potassium persulphate and allowing the mixture to stand in the dark at room temperature for 16 h before use. The ABTS radical cation solution was diluted to an absorbance of 0.70±0.02 at 734 nm and equilibrated at 30°C for 30 min. Each sample (0.2 ml) with various concentrations (1, 3, 10, 30, 100, 300 and 1000 µg/ml) was mixed with 2.0 ml of diluted ABTS radical cation solution. After reaction at room temperature for 6 min, the absorbance at 734 nm was measured immediately. The ABTS scavenging effect was calculated as follows:

ABTS scavenging effect (%) = $[A_0-(As-Ab)]/A_0 \times 100$

where $A_0$:$A_{734}$ of ABTS without sample, As: $A_{734}$ of sample and ABTS, and Ab: $A_{734}$ of sample without ABTS.

#### DPPH radicals scavenging assay

DPPH radical cation scavenging activity of berberine hydrochloride was carried out as described by Luo et al. (2009, 2011) and Braca (2001) with minor modifications. Vitamin C and BHT were used as reference material. 3 ml of sample with various concentrations (10, 30, 100, 300, 1000, 2000, 3000 and 4000 µg/ml) was added to 1 ml of 0.1 mM solution of DPPH. The solution was kept at room temperature for 30 min, and the absorbance at 517 nm was measured. The DPPH scavenging effect was calculated as follows:

DPPH scavenging effect (%) = $[A_0-(As-Ab)]/A_0 \times 100$

where $A_0$ is the absorbance of DPPH without sample, As is the absorbance of sample and DPPH, and Ab is the absorbance of sample without DPPH.

#### Hydroxyl radical scavenging assay

The hydroxyl radical scavenging activity of berberine hydrochloride was carried out according to the method of Luo et al. (2010, 2011) and Wang et al. (2008), with some modifications. Various concentrations (1, 3, 10, 30, 100, 300 and 1000 µg/ml) samples were incubated with 2.0 mM EDTA-Fe (0.5 ml), 3% $H_2O_2$ (1.0 ml) and 360 µg/ml crocus in 4.5 ml sodium phosphate buffer (150.0 mM, pH 7.4) for 30 min at 37°C, and hydroxyl radical was detected by monitoring absorbance at 520 nm. The hydroxyl radical scavenging effect was calculated as follows:

Hydroxyl radical scavenging effect (%) = $[(A_0-As) / A_0] \times 100$

where As is the absorbance of sample and $A_0$ is the absorbance of control. In the control, sample was substituted with distilled water, and sodium phosphate buffer replaced $H_2O_2$.

#### Statistical analysis

All data are expressed as means ± SD (n=3). Data were analyzed by an analysis of variance ($P < 0.05$) and the results were processed by SPSS software.

## RESULTS AND DISCUSSION

### Effect of reducing power

Research has revealed that there is a direct correlation between antioxidant activities and reducing power (Yildirim et al., 2001). To measure reductive power of berberine hydrochloride, we investigated the $Fe^{3+}$-$Fe^{2+}$ transformation in the presence of different concentrations sample, BHT and Vc were used as reference material. The reductive capabilities of berberine hydrochloride and reference material were exhibited as Figure 1. From the result, reducing powers of all samples were in a concentration-dependent manner. The references exhibited strong reducing power, especially for Vc. The reducing power of berberine hydrochloride was also strong, at the high concentrations (from 1000 to 3000 µg/ml), which was close to BHT ($p < 0.05$).

### Effect of scavenging superoxide radicals

Superoxide radical is known to be a very harmful species and plays an important role in the formation of other reactive oxygen-species such as hydroxyl radical, hydrogen peroxide, or singlet oxygen in living systems (Li

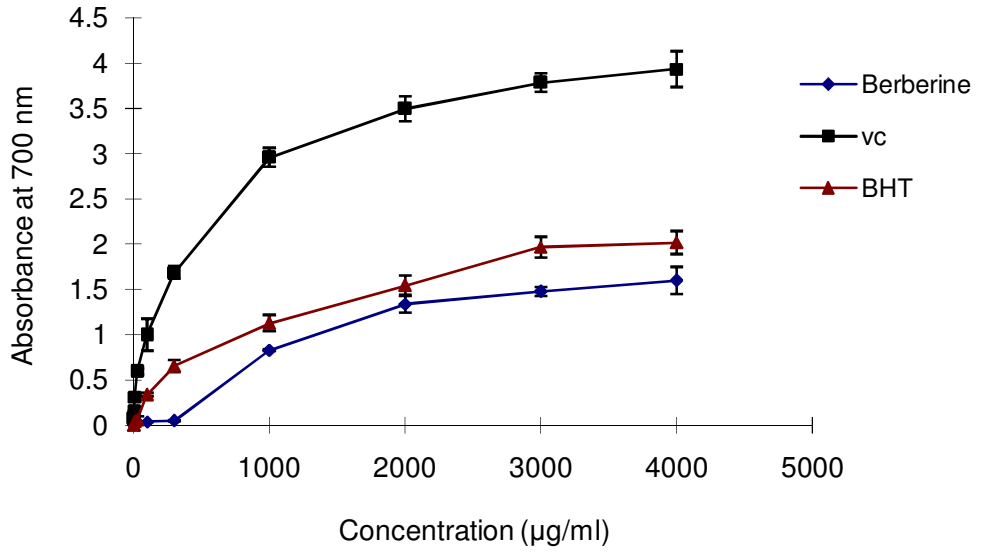


**Figure.1.** Reductive abilities of Berberine and the references. Results are presented as means ± standard deviations.

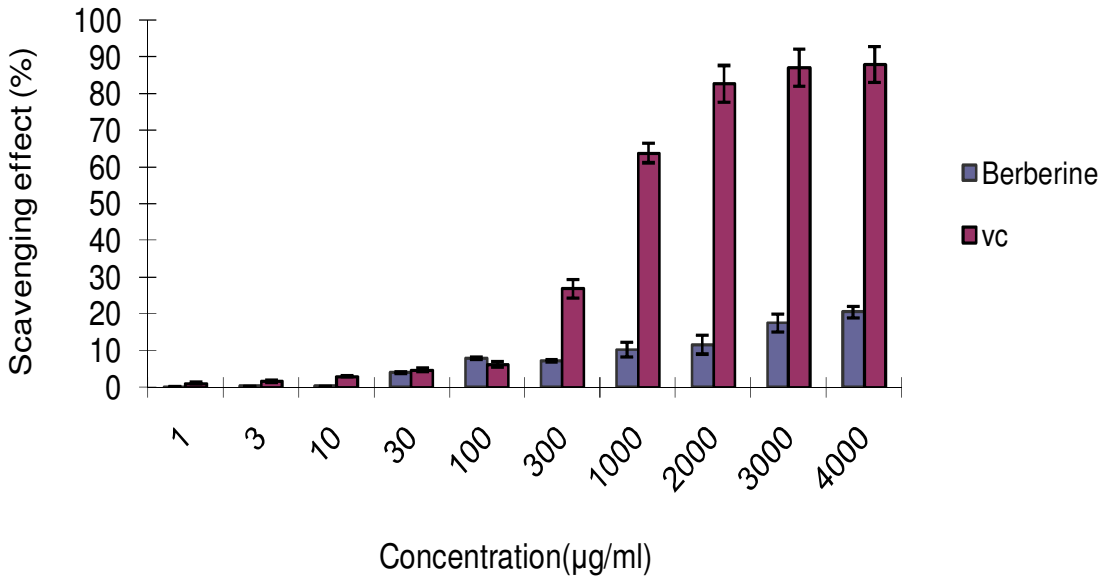


**Figure.2.** The superoxide radicals scavenging activities of Berberine and Vc. Results are presented as means ± standard deviations.

et al., 2009). Figure 2 shows the superoxide scavenging activity of berberine hydrochloride was increased with the increase of concentration, while it was not significantly active at different concentrations. At the concentration of 4000 μg/ml, the scavenging rates of vitamin C and berberine hydrochloride reached 87.99 and 20.53%, respectively. The results indicated that berberine hydrochloride exhibited very low superoxide radical scavenging activity.

**Effect of scavenging ABTS radicals**

The ABTS assay is often used in evaluating total antioxidant ability in both lipophilic and hydrophilic samples. The results of scavenging power on ABTS free radical for the present experiment were shown in Figure 3. The scavenging power of berberine hydrochloride was strong at the range of 300 to 1000 μg/ml, which was closed to that of BHT, though that was lower than vitamin

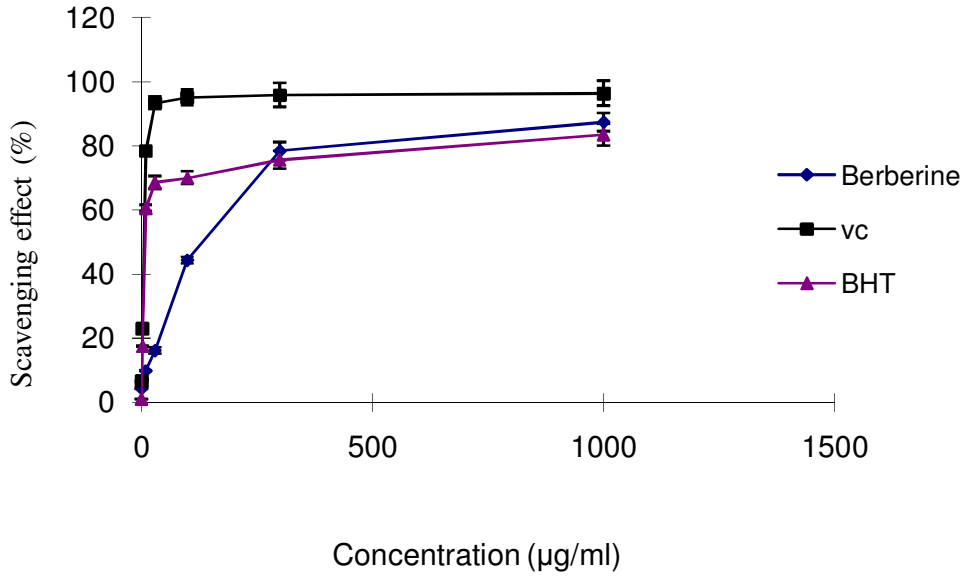


**Figure 3.** The ABTS scavenging activity of berberine and the references. Results are presented as means ± standard deviations.

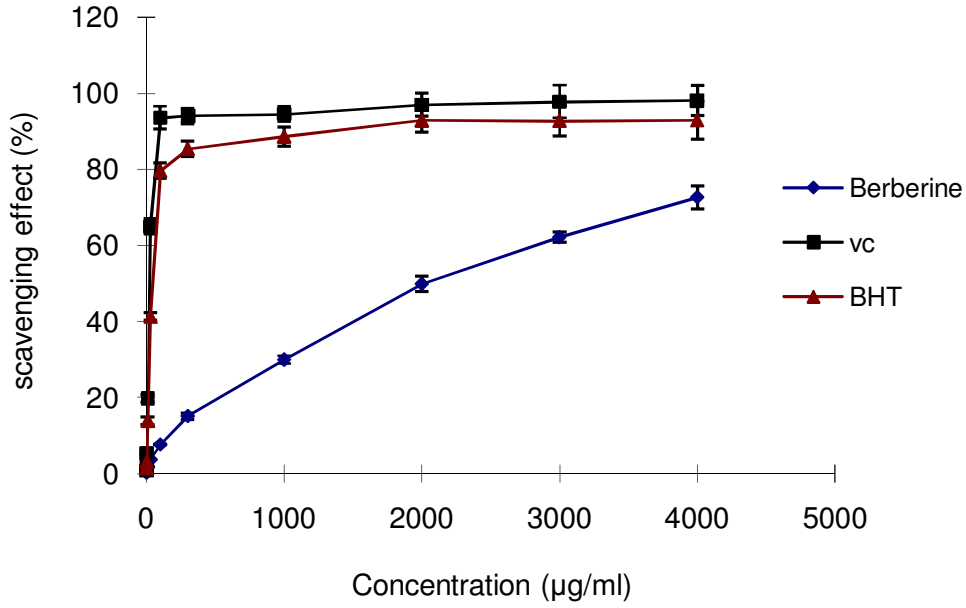


**Figure 4.** The DPPH scavenging activity of berberine and the references. Results are presented as means ± standard deviations.

C. The results indicated that berberine hydrochloride had excellent ABTS radical scavenging activity and should be explored as novel potential antioxidants.

## Effect of scavenging DPPH radicals

In this experiment, the scavenging ability of berberine hydrochloride on DPPH free radical were examined in the concentration range of 10 to 4000 µg/ml using the DPPH colorimetric assay. And the results were given in Figure 4. As is illustrated in the figure, all the samples obvious scavenging activity in a concentration dependent manner.The results indicated that Berberine hydrochloride exhibited very significantly radical scavenging. The effect of Berberine hydrochloride was

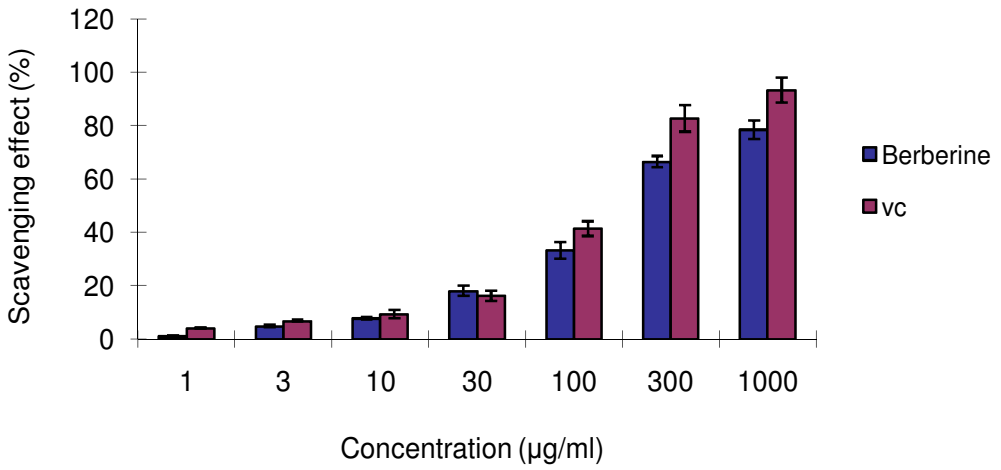


**Figure 5.** The hydroxyl radicals scavenging activity of berberine and the references. Results are presented as means ± standard deviations.

strong (72.72% at the high dose 4000 µg/ml), but lower than that of BHT (92.97% at the high dose 4000 µg/ml). The results suggest that berberine hydrochloride display scavenging effect on DPPH radicals generation that could help prevent or ameliorate oxidative damage.

### Effect of scavenging hydroxyl radicals

Hydroxyl radicals were mainly responsible for the oxidative injury of biomolecules. The scavenging ability of berberine hydrochloride compared to those of vitamin C was shown in Figure 5. The samples exhibited obvious scavenging activities on hydroxyl radical in a concentration-dependent manner. The scavenging activities of berberine hydrochloride increased very significantly with increasing concentrations (300 to 1000 µg/ml). Especially in the high doses (1000 µg/ml), berberine hydrochloride exhibited very high radical scavenging, which was close to that of Vitamin C ($p<0.05$). So, it was obvious that berberine hydrochloride has significant effects on hydroxyl radicals scavenging.

## Conclusion

In the present study, antioxidant ability *in vitro* of berberine hydrochloride was studied. According to the results above, it was concluded that berberine hydrochloride has significant radicals scavenging effects, especially on ABTS, hydroxyl radicals and DPPH radicals. Moreover, berberine hydrochloride has strong reductive ability. Therefore, the results of this study clearly indicate that berberine hydrochloride has powerful antioxidant capacity *in vitro* and the capacity was concentration dependent. Various studies have shown that diabetes mellitus is associated with increased formation of free radicals and decrease in antioxidant potential. Due to these events, the balance normally present in cells between radical formation and protection against them is disturbed. This leads to oxidative damage of cell components such as proteins, lipids, and nucleic acids. When the β-cells of the pancreas were destroyed, the secretion of insulin would be abnormal. It would lead to diabetes mellitus. Some researches have indicated berberine hydrochloride could treat the diabetes mellitus through increasing the activity of superoxide dismutase, blocking malondialdehyde, and attenuating glutathione. From the results above, we concluded the other mechanism on treat the diabetes mellitus of berberine hydrochloride. Because of berberine hydrochloride has significant radicals scavenging effects, so it was indicate that berberine hydrochloride has the protective effects against β-cell damage and antioxidant of pancreas in diabetic. Therefore, it seems reasonable that antioxidants can play an important role in the improvement of diabetes and in screening the novel treatment drug of diabetes mellitus.

## ACKNOWLEDGEMENTS

This study was supported by the Science and Technology Fund of Dujiangyan (2007-61) and the Research Fund of Sichuan Education Department (08ZD036).

### REFERENCES

Braca A, Tommasi ND, Di Bari L, Pizza C, Politi M, Morelli I(2001). Antioxidant principles from *Bauhinia tarapotensis*. J. Nat. Proc., 64: 892-895.

Esra K, Müberra K, Erdem Y, K.Hüsnü CB(2002). A comparative study on the anti-inflammatory, antinociceptive and antipyretic effects of isoquinoline alkaloids from the roots of Turkish *Berberis* species. Life Sci., 72(6): 645-657.

Fan YJ, He XJ, Zhou SD, Luo AX, He T, Chun Z (2009). Composition analysis and antioxidant activity of polysaccharide from *Dendrobium denneanum*. Int. J. Biol. Macromol., 45: 169-173.

Fukuda K, Hibiya Y, Mutoh M, Koshiji M, Akao S, Fujiwara H (1999). Inhibition by berberine of cyclooxygenese-2 transcriptional activity in human colon cancer cells. J. Ethnopharm., 66(2):227-233.

Iwasa K, Lee DU, Kang SI, Wiegrebe W (1998). Antimicrobial activity of 8-alkyl-and 8-phenyl-substituted berberines and their 12-bromo-derivatives. J. Nat. Proc., 61: 1150-1153.

Li HY, Hao ZB, Wang XL, Huang L, Li JP (2009). Antioxidant activities of extracts and fractions from *Lysimachia foenum-graecum* Hance. Bioresour. Technol., 100: 970-974.

Luo A,X Ge ZF, Fan YJ, Luo AS, Chun Z, He XJ(2011). *In Vitro* and *In Vivo* Antioxidant Activity of a Water-Soluble Polysaccharide from *Dendrobium denneanum*. Molecules. 16: 1579-1592.

Luo AX, Fan YJ, Luo AS (2011). *In vitro* Free Radicals Scavenging Activities of Polysaccharide from *Polygonum Multiflorum* Thunb. J Med. Plants Res., 5(6): 966-972.

Luo AX, He XJ, Zhou SD, Fan YJ, He T, Chun Z (2009). *In vitro* antioxidant activities of a water-soluble polysaccharide derived from *Dendrobium nobile* Lindl. Extracts. Int. J. Biol. Macromol., 45: 359-363.

Luo AX, He XJ, Zhou SD, Fan YJ, Luo AS, Chun Z (2010). Purification, Composition Analysis and Antioxidant Activity of the Polysaccharides from *Dendrobium nobile* Lindl. Carbohydr. Polym., 79: 1014-1019.

Raza F, Gholam AG, Mohammad HHK (2007). Antioxidant activity of various extracts of old tea leaves and black tea wastes (*Camellia sinensis* L.). Food Chem., 100: 231-236.

Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C (1999). Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radic. Biol. Med., 26: 1231-1237.

Tang LQ, Wei W, Chen LM, Liu S (2006). Effects of berberine on diabetes induced by alloxan and a high-fat/high-cholesterol diet in rats. J. Ethnopharm., 108(1): 109-115.

Tatiya AU, Kulkarni AS, Surana SJ and Bari ND(2010). Antioxidant and hypolipidemic effect of *Caralluma adscendens* Roxb. in alloxanized diabetic rats. Int. J. Pharmacol., 6(4): 362-368.

Wang BS, Li BS, Zeng QX, Liu HX (2008). Antioxidant and free radical scavenging activities of pigments extracted from molasses alcohol wastewater. Food Chem., 107: 1198-1204.

Wang J, Zhang QB, Zhang ZS, Li Z (2008). Antioxidant activity of sulfated polysaccharide fractions extracted from *Laminaria japonica*. Int. J. Biol. Macromol., 42: 127-132.

Yen GH, Chen HY (1995). Antioxidant activity of various tea extracts in relation to their antimutagenicity. J. Agric. Food Chem., 43: 27-32.

Yildirim A, Mavi A, Kara AA (2001). Determination of antioxidant and antimicrobial activities of *Rumex crispus* L. extracts. J. Agric. Food Chem., 49: 4083-4089.

Yin J, Xing H, Ye J (2008). Efficacy of berberine in patients with type 2 diabetes mellitus. Metabolism, 57: 712-717.

Zhao YP, Yu WL, Wang DP (2003). Chemiluminescence determination of free radical scavenging abilities of “tea pigments” and comparison with “tea polyphenols.” Food Chem., 80: 115-118.

Zhou H, Mineshita S (2000). The Effect of Berberine Chloride on Experimental Colitis in Rats *In Vivo* and *In Vitro*. J. Pharm. Exp. Ther., 294: 822-829.

Zhou JY, Zhou SW, Tang JL, Zhang KB, Guang LX, Huang YP, Xu Y, Ying Y, Zhang L, Li DD (2009). Protective effect of berberine on beta cells in streptozotocin- and high-carbohydrate/high-fat diet-induced diabetic rats. Eur. J. Pharmacol., 606(1-3): 262-268.