Study on the Antioxidant, Hypoglycemic and Laxative Components of *Cistanche Deserticola*

Liwei Wang, Bing Zhao*
National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences, 100190, Beijing, P. R. China
**Corresponding author E-mail: bzhao@ipe.ac.cn*

Abstract: *Cistanche deserticola* (CD) is a traditional Chinese medicinal herb with multi-functions. However, the component-activity relationship has not been clarified. The antioxidant, hypoglycemic and laxative activities of different components extracted from CD such as phenylethanoid glycosides (PeG), polysaccharide (CDP), oligosaccharide (OLS) and soluble dietary fiber (SDF) were compared in this study based on the methods of reducing power, scavenging DPPH and ABTS radical, α-glucosidase and α-amylase inhibition, small intestinal transit and defecation. Results showed that PeG had higher antioxidant activity than CDP, while SDF was more effective than other CD extracts in the inhibition of both α-glucosidase and α-amylase. Both OLS and SDF have laxative activity, and the former is better. Small intestinal transit was significantly accelerated when the mice were fed with OLS or SDF at middle and high dose ($P < 0.05$). In the defecation time of first black feces and granule number of feces within 6 h, significant difference appeared in the middle dosage of OLS group, while not for SDF group. This result could provide new insight into a full utilization of different CD extracts, which could be useful for treatment of oxidative stress reaction, diabetes, constipation and their complications.
Keywords: *Cistanche deserticola*; antioxidant; hypoglycemic; laxative; component-activity.

1. Introduction

Cistanche deserticola (CD), a parasitic plant, is native to the desert region of north-western China. It is used as a traditional Chinese tonic drug for kidney deficiency, characterized by impotence, pain in the loins and knees, female sterility and constipation due to dryness of the bowel in the senile. The plant is called 'desert ginseng' by local inhabitants because of its ginseng-like tonic effects. Recent studies revealed that CD had antioxidants, anti-inflammatory[1,2], antifatigue[3] and immunomodulatory activities[4]. Phenylethanoid glycosides (PeG) and polysaccharides were major active constituents in CD, and there are some literatures about their antioxidant activity. For example, he reported the antioxidant activity of PeG from *Brandisia hancei*[5]. Cheng also found that there was a correlation between high antioxidant activity and PeG accumulation in the cell suspension cultures of CD[6]. Sui found a polysaccharide isolated from CD exhibited strong antioxidant activities[7]. However, antioxidant activity between PeG and polysaccharides is still unknown, which is also an urgent problem facing the utilization of CD. Besides, there are also much oligosaccharide and dietary fiber in CD, which were usually left with no ultilization[8]. Oligosaccharide and dietary fiber were reported to have laxative activity[9, 10]. In order to make a full utilization of CD, we investigate the antioxidant, hypoglycemic and laxative activity of each component in CD.

2. Materials and methods

2.1 Plant material

CD was provided by Alashan Cistanche Group Co., Ltd in Inner Mongolia. A voucher specimen was deposited in National Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences.

2.2 Chemicals and reagents

α-glucosidase and α-amylase were purchased from Sigma-Aldrich (St. Louis, MO). PeG with the purity of 40%, 60% and 80% were purchased from Hetian Dichen Biotech Co., Ltd (Hetian, Xinjiang, China). Ultrasonic circulating extraction equipment (TGCXZ-20B, Beijing Hong Xiang Long Co., Ltd.) designed by our laboratory was employed in this study. All other reagents used were of analytical reagent grade.

2.3 Preparation of CD extracts

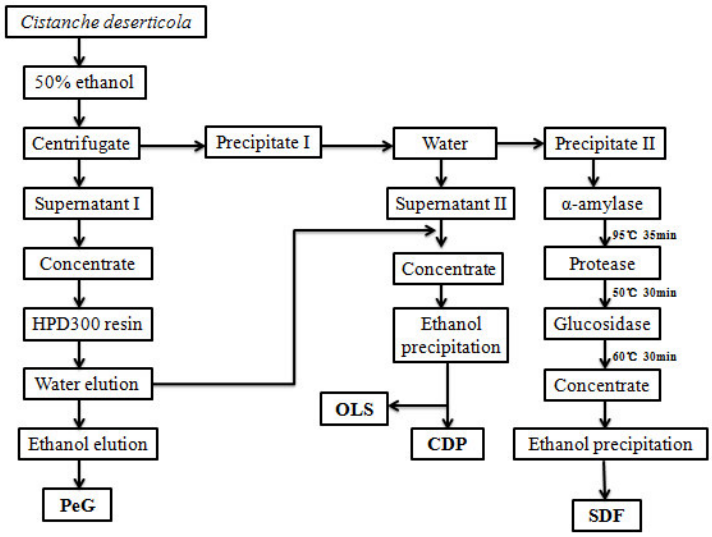

Figure 1. Extraction chart of different components in CD

The dried CD material (1 kg) was powdered and extracted by ultrasonic extraction with 20 L 50% ethanol solution, then centrifugated to get supernatant I and precipitate I. Supernatant I was evaporated under reduced pressure with a rotary evaporator and then added into HPD300 resin. The column was first washed by deionized water and then eluted by 50% ethanol solution, the later was dried to get PeG[11]. Precipitate I was then extracted with 10 L water, centrifugated to get supernatant II and precipitate II. Water solution from HPD300 and supernatant II were blended and concentrated. Then four volume of ethanol was added into the concentrate, and the whole solution was centrifugated after 24 h. The precipitate was *Cistanche deserticola* polysaccharide (CDP) and the resulting solution was then concentrated to get oligosaccharide (OLS). Precipitate II was dissolved in phosphate buffer (pH 8.2, 0.1 M) with the ratio of 1:20, then α-amylase, neutral protease, and glucosidase were added with enzymatic hydrolysis of 95 ℃(35 min), 50 ℃ (30 min), 60 ℃ (30 min), respectively. Enzymatic hydrolysate liquid was then concentrated and four volume of ethanol was added for 24 h to get soluble dietary fiber (SDF). The extraction chart of different components in CD was shown in Figure1.

2.4 Determination of PeG

The standard curve of PeG content was measured using echinacoside as the standard at 333 nm using an ultraviolet spectrophotometer[11].

2.5 Antioxidant activity

Antioxidant activity of CD components was evaluated by reducing power, scavenging DPPH radical [12] and ABTS radical. ABTS radical scavenging test was based on spectrophotometric measurement of the change in absorbance at 734 nm[13-15]. 50 ml ABTS stock solution (7 mmol/L) and 880 μl potassium persulfate solution (140 mmol/L) were mixed to prepare the ABTS radical solution, and the solution was placed in dark for 24 h. Then ABTS radical solution was diluted to an absorbance of 0.7±0.02. A solvent of the sample was used as a control. ABTS radical scavenging activity was calculated as follows:

$$\text{ABTS radical scavenging } (\%) = \frac{A_{control} - A_{sample}}{A_{control}} \times 100$$

2.6 Hypoglycemic activity

2.6.1 Assay for α-glucosidase inhibitory activity

α-glucosidase inhibition of CD extracts were determined as previously reported with some modification[16]. Briefly, 50 μl diluted sample, 50 μl phosphate buffer (0.2 M, pH 6.8) , 50 μl 5 mM p-nitrophenyl-a-D-glucopyranoside (pNPG) solution and 50 μl a-glucosidase solution (pH 6.8, 0.2 U/ml, in 0.2 M phosphate buffer) were incubated in 96-well plates at 37 ℃ for 20 min, then 50 μl sodium carbonate (0.2 M) was added. The absorbance at 405 nm was recorded with a microplate reader. The inhibitory activity calculated as follows:

$$\text{α-glucosidase inhibitory activity (\%)} = (1 - \frac{A_3 - A_4}{A_1 - A_2}) \times 100$$

Where A_1 is the absorbance of control, A_2 is the absorbance of control without pNPG, A_3 is the absorbance of sample, and A_4 is the absorbance of sample without pNPG.

2.6.2 Assay for α-amylase inhibitory activity

The α-amylase inhibition assay was slightly modified according to the literature procedure[17]. α-amylase was dissolved in 0.1 M phosphate buffer (pH 6.8). Various concentrations of sample were added to a solution containing 1% starch and phosphate buffer. The reaction was initiated by adding amylase (1 U/ml) to the incubation medium to a final volume of 1 ml. After 10 min the reaction was stopped by adding 2 ml dinitrosalicylic (DNS) reagent (1% 3,5-dinitrosalicylic acid, 0.2% phenol, 0.05% Na_2SO_3, 1% NaOH, 20% potassium sodium tartrate in aqueous solution) to the reaction mixture. Mixtures were heated at 100 °C for 5 min to develop a yellow-brown color, then cooled to room temperature, and added to a volume of 10 ml by distilled water. Finally, absorbance was recorded at 540 nm using a spectrophotometer. The inhibitory activity calculated as follows:

$$\text{α-amylase inhibitory activity (\%)} = (1 - \frac{A_1 - A_2}{A_0}) \times 100$$

Where A_0 is the absorbance of control, A_1 is the absorbance of sample and A_2 is the absorbance of sample without α-amylase.

2.7 Experimental animals

160 eight-week-old ICR mice with the body weight of 35±3 g were purchased from Vital River Laboratory Animal Technology Co. Ltd. All animals' treatments were strictly in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. The animals were kept at 24±2 ℃ with the humidity of 50±5%.

2.8 Laxative activity

2.8.1 Effect on small intestinal transit

The animals were divided into eight groups with ten mice in each group. Groups were set as control group, model group and sample group, with OLS and SDF as the samples at doses of 0.5, 1.5 and 4.5 g/kg·bw, respectively. The control group and model group were fed with normal diet, while sample groups were fed with OLS and SDF by intragastric administration for 14 days besides the normal diet. Then all the mice were fasted overnight with free access to water. The control group was administered with distilled water, and the other groups received compound diphenoxylate tablets (10 mg/kg·bw). After 30 min, all the mice were fed with the small intestinal transit indicator containing 5% charcoal and 10% acacia gum in water. The mice were then sacrificed after feeding with the indicator for 25 min. Their small intestines were removed, and the length of the small intestines and the distance of charcoal transit were measured[18]. The percentage of small intestinal transit was calculated as follows:

$$\text{small intestinal transit (\%)} = \frac{\text{distance of the charcoal traveled}}{\text{length of the small transit}} \times 100$$

2.8.2 Effect on defecation

The method of animal model was in accordance with 2.6.1. The defecation time of the first black feces and granule number of feces within 6 h were recorded after intragastric administration of the activated carbon[19].

2.9 Statistical analysis

All data obtained during the experiment were expressed in terms of mean and standard errors, and further analyzed by ANOVA one-way analysis of variance. When probability (P) was less than 0.05, the difference was considered to be significant.

3. Results and discussion

3.1 Phytochemical analysis of CD extracts

After removal of the solvents using freeze drying method, the final yields of the PeG, OLS, CDP and SDF were 4.53, 15.08, 3.65 and 5.10%, respectively. The dry materials of PeG, OLS and CDP were brown, while SDF was with grey color. The PeG purity is 76.58%. The total content of OLS and SDF is much higher than that

of PeG and CDP, while the latter were usually used. Therefore, other activities are needed to develop the use of CD.

3.2 Antioxidant activity

Oxidative stress may contribute to the development of several age-related and chronic diseases such as cancer, diabetes, neurodegenerative, and cardiovascular diseases. Natural food-derived components have received great attention in the last two decades for the antioxidant potential[20, 21]. Therefore, antioxidant activities of the CD extracts were determined by the reducing power test, scavenging DPPH radical and ABTS radical test. The results are shown in Figure 2.

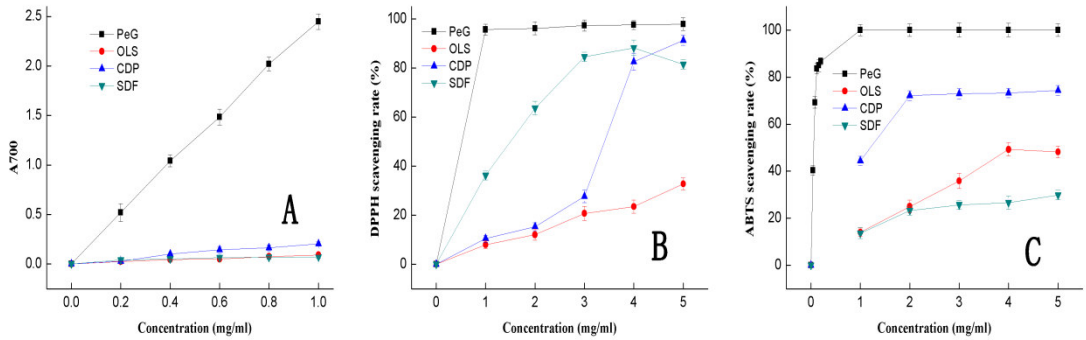

Figure 2. Antioxidant activities of CD extracts

A: reducing power, B: DPPH scavenging rate, C: ABTS scavenging rate

PeG, CDP and OLS showed different ferric-reducing powers, and the absorbance in 700 nm decreased in the same order. At the concentration of 1 mg/ml, the value of SDF was 0.07, which was much lower than those of other CD extracts. As PeG has the highest antioxidant activity, different PeG with the purity of 40%, 60% and 80% were also tested, and the results showed that values in 700 nm increased with increasing PeG purity (Figure 3).

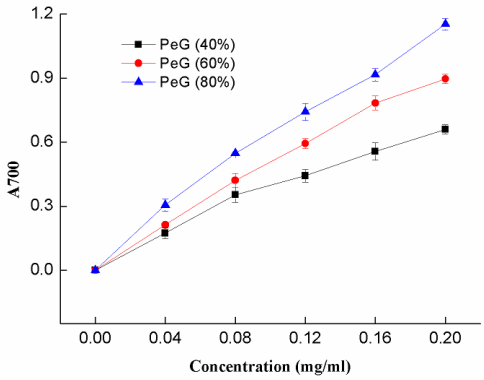

Figure 3. Reducing power of PeG with different purity

The scavenging effects of CD extracts on the DPPH radical increased dependent on concentration. PeG has the strongest antioxidant activity, the scavenging effect of which was 95.61% at 1 mg/ml. Scavenging activity of CDP and OLS were much lower at the concentration of less than 3 mg/ml. However this scavenging activity reached 91.31% and 32.77% respectively at the concentration of 5 mg/ml. SDF is more effective than OLS at the same concentration, which is a little different from that of reducing power test.

The result of the ABTS test showed a similar trend with reducing power test. The ABTS radical-scavenging activity decreased in the order of PeG>CDP>OLS>SDF. So far, many studies have investigated the antioxidant activities of polysaccharides from plants, and most of them found that plant polysaccharides have high antioxidant activities[22-24]. However, according to our study, the antioxidant activity of PeG is higher than CDP.

3.3 Hypoglycemic activity

α-amylase and α-glucosidase inhibition assay are models of hypoglycemic activity in vitro[25, 26]. *Cistanche tinctoria* was reported to have antidiabetic activity. However, the component was limited to aqueous extract[27]. CD, which also belongs to Cistanche, might have the same activity. Therefore, the CD extracts were investigated for a-glucosidase and α-amylase inhibition. As shown in Figure 4, significant difference was found among the

four kinds of CD extracts, and SDF possessed the highest inhibition, especially in α-amylase inhibition. In α-glucosidase inhibitory test, the activity decreased in the order of SDF>CDP>OLS, while PeG did not show any effect on a-glucosidase inhibition. However, in α-amylase inhibitory test, PeG has higher activity than that of CDP and OLS. This may be contributed to the different structure of enzymes. Phenylethanoid glycosides were reported to possess hypoglycemic activity and improve glucose tolerance in starch-loaded mice[28]. In this study, SDF revealed to have higher hypoglycemic activity than PeG, and could be a promising alternative therapy for preventing diabetic and its complications, which is also a comprehensive utilization of CD.

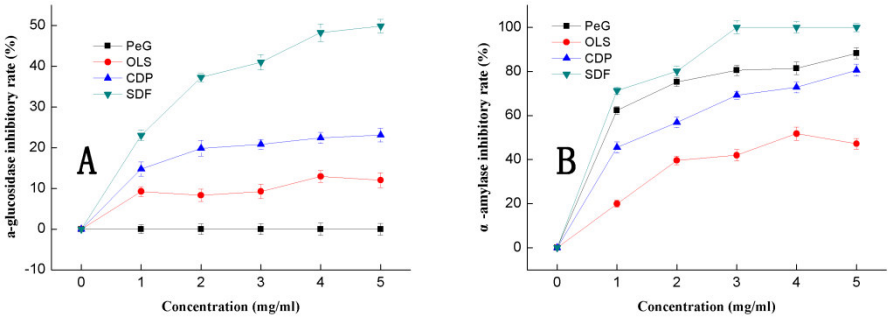

Figure 4. Hypoglycemic activities of CD extracts
A: α-glucosidase inhibition, B: α-amylase inhibition

3.4 Laxative activity

In this study, we evaluated the laxative activity of OLS and SDF by small intestinal transit and defecation model, and the results were shown in Table 1.

Table 1. Small intestinal transit and defecation of OLS and SDF

Group	Dosage (g/kg·bw)	Active carbon propelling rate (%)	First black feces (min)	granule number in 6h
Control	-	87.0±8.7	154±19.2	12.8±2.0
Model	-	43.2±7.2 [a]	286.0±10.7 [a]	5.7±1.0 [a]
OLS-low	0.5	45.6±7.0	250.1±16.6	7.1±1.1
OLS-middle	1.5	64.6±7.2 [b]	166.3±17.3 [b]	12.8±1.3 [b]
OLS-high	4.5	75.1±5.3 [b]	154.6±21.0 [b]	15.2±1.7 [b]
SDF-low	0.5	46.3±6.7	251.0±40.3	6.3±1.4
SDF-middle	1.5	68.4±8.1 [b]	216.2±31.4	6.5±0.9
SDF-high	4.5	73.9±5.6 [b]	209.6±16.3 [b]	11.1±1.7 [b]

a: compared with control group, $p < 0.05$, b: compared with model group, $p < 0.05$.

In small intestinal transit test, the active carbon propelling rate in constipation model group was significantly lower than that in control group, which suggested modeling success. Small intestinal transit was significantly accelerated when the mice were fed with OLS or SDF at middle and high dose in comparison with the constipation model ($P < 0.05$).

In defecation test, compared with control group, the defecation time of the first black feces in model group was significantly longer ($P < 0.05$), and the granule number of feces within 6 h was significantly decreased ($P < 0.05$). Compared with the model group, the defecation time of the first black feces in OLS group and SDF group were shortened, and the granule number of feces within 6 h increased in the middle and high dose group of OLS ($P < 0.05$), and high dose group of SDF ($P < 0.05$). Taken together, these results demonstrate that both OLS and SDF have laxative activity, and compared with SDF, OLS has higher laxative activity.

4. Conclusion

In conclusion, the four kinds of CD extracts showed different activities among antioxidant, hypoglycemic and laxative tests. PeG has higher antioxidant activity than CDP. However, SDF is more effective than PeG in the inhibition of both α-glucosidase and α-amylase. Both OLS and SDF have laxative activity. In defecation time of the first black feces and granule number of feces within 6 h, significant difference appeared in the middle dosage of OLS group, while not for SDF group, which suggested OLS have better laxative activity than that of SDF. Thus, this study provides basis for the medicinal use of CD in disorders, such as ageing, diabetes and constipation.

5. References

[1] Nan, Z.D., Zeng, K.W., Shi, S.P., Zhao, M.B., Jiang, Y., Tu, P.F. Phenylethanoid glycosides with anti-inflammatory activities from the stems of *Cistanche deserticola* cultured in Tarim desert. Fitoterapia. 2013, 89: 167-174.

[2] Lin, L.W., Hsieh, M.T., Tsai, F.H., Wang, W.H., Wu, C.R. Anti-nociceptive and anti-inflammatory activity caused by *Cistanche deserticola* in rodents. J. Ethnopharmacol. 2002, 83(3): 177-182.

[3] Cai, R.L., Yang, M.H., Shi, Y., Chen, J., Li, Y.C., Qi, Y. Antifatigue activity of phenylethanoid-rich extract from *Cistanche deserticola.* Phytother. Res. 2010, 24(2): 313-315.

[4] Dong, Q., Yao, J., Fang, J.N., Ding, K. Structural characterization and immunological activity of two cold-water extractable polysaccharides from *Cistanche deserticola* Y. C. Ma. Carbohydr. Res. 2007, 342(10): 1343-1349.

[5] He, Z.D., Lau, K.M., Xu, H.X., Li, P.C., Pui-Hay, B.P. Antioxidant activity of phenylethanoid glycosides from *Brandisia hancei.* J. Ethnopharmacol. 2000, 71(3): 483-486.

[6] Cheng, X., Wei, T., Guo, B., Ni, W., Liu, C.Z. *Cistanche deserticola* cell suspension cultures: phenylethanoid glycosides biosynthesis and antioxidant activity. Process. Biochem. 2005, 40(9): 3119-3124.

[7] Sui, Z.F., Gu, T.M., Liu, B., Peng, S.W., Zhao, Z.L., Li, L., Shi, D.F., Yang, R.Y. Water-soluble carbohydrate compound from the bodies of Herba Cistanches: isolation and its scavenging effect on free radical in skin. Carbohydr. Polym. 2011, 85(1): 75-79.

[8] Wang, L., Ding, H., Yu, H., Han, L., Lai, Q., Zhang, L., Song, X. Cistanches herba: chemical constituents and pharmacological effects. Chinese. Herb. Med. 2015, 7(2): 135-142.

[9] Wang, J., Huang, H.H., Cheng, Y.F., Yang, G.M. Structure analysis and laxative effects of oligosaccharides isolated from bananas. J. Med. Food. 2012, 15(10): 930-935.

[10] Sturtzel, B., Elmadfa, I. Intervention with dietary fiber to treat constipation and reduce laxative use in residents of nursing homes. Ann. Nutr. Metab. 2008, 52(1): 54-56.

[11] Liu, B., Ouyang, J., Yuan, X., Wang, L., Zhao, B. Adsorption properties and preparative separation of phenylethanoid glycosides from *Cistanche deserticola* by use of macroporous resins. J. Chromatogr. B. 2013, 937: 84-90.

[12] Wang, L.W., Liang, J., Wang, X.D., Yuan, X.F., Zhao, B., Yang, Y.W. High efficient antioxidant activity of extracts from *Lepidium meyenii* Walp. Asian. J. Chem. 2012, 24(10): 4795-4798.

[13] Zhang, D.Y., Wan, Y., Xu, J.Y., Wu, G.H., Li, L., Yao, X.H. Ultrasound extraction of polysaccharides from mulberry leaves and their effect on enhancing antioxidant activity. Carbohydr. Polym. 2016, 137: 473-479.

[14] Przeor, M., Flaczyk, E. Antioxidant properties of paratha type flat bread enriched with white mulberry leaf extract. Indian. J. Tradit. Know. 2016, 15(2): 237-244.

[15] M'Hiri, N., Veys-Renaux, D., Rocca, E., Ioannou, I., Boudhrioua, N.M., Ghoul, M. Corrosion inhibition of carbon steel in acidic medium by orange peel extract and its main antioxidant compounds. Corros. Sci. 2015, 102: 55-62.

[16] Lu, Z., Tu, Z.C., Tao, Y., Hui, W., Xing, X., Fu, Z.F. Antioxidants and α-glucosidase inhibitors from *Ipomoea batatas* leaves identified by bioassay-guided approach and structure-activity relationships. Food. Chem. 2016, 208: 61-67.

[17] Adisakwattana, S., Chantarasinlapin, P., Thammarat, H., Yibchokanun, S. A series of cinnamic acid derivatives and their inhibitory activity on intestinal alpha-glucosidase. J. Enzyme. Inhib. Med. Chem. 2009, 24(5): 1194-1200.

[18] Hsieh, S.K., Xu, J.R., Lin, N.H., Li, Y.C., Chen, G.H., Kuo, P.C., Chen, W.Y., Tzen, J.T.C. Antibacterial and laxative activities of strictinin isolated from Pu'er tea (*Camellia sinensis*). J. Food. Drug. Anal. 2016, 24(4):

722-729.
[19] Najeeb-Ur-Rehman, Mehmood, M.H., Alkharfy, K.M., Gilani, A.H. Prokinetic and laxative activities of *Lepidium sativum* seed extract with species and tissue selective gut stimulatory actions. J. Ethnopharmacol. 2011, 134(3): 878-883.
[20] Kregel, K.C. Caloric restriction, aging and oxidative stress. Sci. 2014, 273(5271): 59-63.
[21] Rahal, A., Kumar, A., Singh, V., Yadav, B., Tiwari, R., Chakraborty, S., Dhama, K. Oxidative stress, prooxidants, and antioxidants: the interplay. Biomed. Res. Int. 2014, 2014: 1-19.
[22] Zha, S., Zhao, Q., Chen, J., Wang, L., Zhang, G., Zhang, H., Zhao, B. Extraction, purification and antioxidant activities of the polysaccharides from maca (*Lepidium meyenii*). Carbohydr. Polym. 2014, 111: 584-587.
[23] Fan, J., Feng, H., Yu, Y., Sun, M., Liu, Y., Li, T., Sun, X., Liu, S., Sun, M. Antioxidant activities of the polysaccharides of *Chuanminshen violaceum.* Carbohydr. Polym. 2016, 157: 629-636.
[24] Zhou, X., Wang, H., Wang, B., Liang, F., Ming, Y., Jing, L., Zhou, L., Ding, C. Characterization and antioxidant activities of polysaccharides from the leaves of *Lilium lancifolium* Thunb. Int. J. Biol. Macromol. 2016, 92: 148-155.
[25] Barapatre, A., Aadil, K.R., Tiwary, B.N., Jha, H. In vitro antioxidant and antidiabetic activities of biomodified lignin from *Acacia nilotica* wood. Int. J. Biol. Macromol. 2015, 75: 81-89.
[26] Ali, M.Y., Jung, H.J., Jannat, S., Jung, H.A., Choi, J.S. In vitro antidiabetic and antioxidant potential of the ethanolic extract of skipjack tuna (*Katsuwonus Pelamis*) heart. J. Food. Biochem. 2016, 40(4): 583-592.
[27] Bouzitouna, A., Ouali, K., Djeddi, S. Protective effects of *Cistanche tinctoria* aqueous extract on blood glucose and antioxidant defense system of pancreatic β-cells in experimental diabetes in rats. Int. J. Pharm. Sci. Rev. Res. 2015, 32(2): 243-249.
[28] Morikawa, T., Ninomiya, K., Imamura, M., Akaki, J., Fujikura, S., Pan, Y., Yuan, D., Yoshikawa, M., Jia, X., Li, Z. Acylated phenylethanoid glycosides, echinacoside and acteoside from *Cistanche tubulosa*, improve glucose tolerance in mice. J. Nat. Med. 2014, 68(3): 561-566.