academicJournals

Vol. 7(19), pp. 1175-1178, 22 May, 2013
DOI 10.5897/AJPP12.225
ISSN 1996-0816 © 2013 Academic Journals
http://www.academicjournals.org/AJPP

African Journal of Pharmacy and Pharmacology

Full Length Research Paper

Lycium barbarum polysaccharides (LBP) extraction technology and its antioxidation activity

Ling Yuan[1,2], Yi Nan[3], Rong Wang[3] and Yang Niu[3]*

[1]Pharmacy College of Ningxia Medical University, Yinchuan, Ningxia Province.750004, People's Republic of China.
[2]Minority Traditional Medical Center of Minzu University of China, Beijing.100081, People's Republic of China.
[3]Traditional Chinese Medicine College of Ningxia Medical University, Yinchuan City, Ningxia Province, 750004, People's Republic of China.

Accepted 7 May, 2012

This study was carried out to investigate the technology of extracting *Lycium barbarum* polysaccharide (LBP) and its antioxidation activity, using orthogonal experimental method to optimize its extraction conditions. Fenton reaction and pyrogallol experimental method were adopted to determine the antioxidation activity of LBP. The three factors investigated in the orthogonal experiment affected the results. Different concentrations of LBP effectively cleared hydroxyl radical (OH^-) and superoxide anion (O_2^+). It was concluded that the extraction of LBP by cold maceration as well as the three factors, (solvent volume, extraction time and extraction times) affects its yield, and that it has antioxidation activity.

Key words: *Lycium barbarum* polysaccharides, antioxidation activity.

INTRODUCTION

Barbary wolfberry is a beneficial traditional Chinese medicinal food. Barbary wolfberry, belonging to Solanaceae family, *Lycium*, is a perennial deciduous shrub, has more than 80 species in the whole world and is widely distributed mainly in North and South America and Eurasia. In China, it is mainly distributed in Gansu, Ningxia, Tianjin and other places. Ningxia wolfberry is considered the best (He et al., 1997). Barbary wolfberry contains a variety of active ingredients, including carbohydrate, amino acids, trace elements, vitamins, alkaloids, fatty acids, etc; of it species, *Lycium barbarum* polysaccharide (LBP) has the highest carbohydrate content and its total carbohydrate content is up to 46.5% (Jiangsu, 1977).

In modern pharmacology, it is confirmed that polysaccharides plants clear oxygen radicals, increase antioxidase activity, resist lipid peroxidation, reduce the production of PGs, LTs, malonaldehyde (MDA) and other lipid peroxidation metabolites. They also do not allow cell metabolism and morphological changes caused by molecular aggregation and cross linking of membrane proteins and enzymes; and finally they clear free radicals and delay senility. Some studies (Dai et al., 2010) show that barbary wolfberry can reduce serum MDA content in elderly mouse and increase the biological activity of superoxide dismutase (SOD). This indicates that it can effectively increase antioxidase activity, clear oxygen radicals and reduce lipid peroxidation level. That shows it has good antioxidation activity.

This experiment extracts, separates and purifies LBP, to prove its antioxidation activity and explore its action mechanism.

*Corresponding author. E-mail: nanyiailing10@sohu.com. Tel: +86 0951-4091605.

Table 1. Investigation of the levels of the factors for LBP extraction technology.

Level	Factor 1	Factor 2	Factor 3
	Extraction time (h) A	Extraction times B	Solvent volume (ml/g) C
1	1	1	40
2	2	2	60
3	3	4	80

MATERIALS AND METHODS

Kunming mice with body weight of 20 ± 2 g were bought from the Institute of Laboratory Animal Sciences of CAMS; the needed reagents were domestically obtained and were of analytical grade.

Investigation of LBP extraction technology

Pretreatment

Ningxia wolfberry was dried at 60°C to constant weight, crushed and screened through a 100 mm diameter-mesh. The filtrate was stored for later use.

Orthogonal experimental design for LBP water extraction technology

LBP is a water-soluble polysaccharide, and its content in Barbary wolfberry is above 35%. It os mainly composed of arabinose, galactose, glucose, rhamnose, mannose and xylose (Huang et al., 1998). In order to increase LBP extraction efficiency, this experiment investigates the three main factors affecting the results namely, solvent volume, extraction time and extraction times. The levels of the factors are shown in Table 1. To precisely weigh 9 shares of sample and number them, the experiment was carried out in a 3 × 3 orthogonal design and LBP content was calculated.

Hydroxyl radical (OH^-) clearing effect (Jin et al., 1996)

H_2O_2/Fe^{2+} system (Chun and David, 2001) is adopted to generate OH^- by Fenton reaction. Precisely weighed 1.5 ml phenanthroline solution (5 mmol/L) was added to 1.0 ml phosphate buffer (pH = 7.4), and simultaneously 0.2 ml 7.5 mmol/L $FeSO_4$ and 1.0 ml 0.1% H_2O_2 were added. Immediately, it was mixed. Water was added to total volume of 10 ml thermostatic water bath at 37°C for 1 h; then the absorbance was measured at 536 nm. In the experiment, LBP extracts were prepared at different concentrations (original herbs had 200 mg, 400 and 600 mg/L) with the aforementioned methods, and A_{536} (sample) was measured. A_{536} (undamaged) was measured without adding sample and H_2O_2. The clearance rate was then calculated.

$$d = [A_{536}\text{ (sample)} - A_{536}\text{ (damaged)}] / [A_{536}\text{ (undamaged)} - A_{536}] \times 100\%$$

Superoxide anion (O_2^+) clearing effect (Zhang et al., 2004)

To adopt pyrogallol autoxidation in order to initiate *in vitro* chemical reaction, 4.5 ml 1 mmol/L Tris-HCl buffer (pH 8.2) was put in a test tube and preheated at 25°C for 20 min; 0.2 ml 3 mmol/L pyrogallol and 2 ml LBP solution of different concentrations were added to water bath at 25°C for accurately 4 min. Then A_{325} value, denoted as A_{sample} was measured. The reaction solution without extract was taken as the reference. A_{325} value, denoted as $A_{damaged}$, was measured and the clearance rate was calculated as follows:

$$\text{Clearance rate} = (A_{damaged} - A_{sample}) / A_{damaged} \times 100\%$$

RESULTS

Orthogonal experiment results

The results show that the amount of water, extraction time and extraction times affect LBP extraction yield. The ranges of the factors are 2.97, 1.16 and 5.65, showing that the impacts on the experimental results are C>A>B. The highest LBP content is 18.66% and the lowest is 12.56%, indicating that $A_3B_1C_3$ (that is, 80 ml/g water, once and 3 h extraction) is the best option (Table 2).

Experimental result for LBP clearing hydroxyl radical (OH^-)

The results in Table 3 show that LBP has significant clearing effect on hydroxy radical (OH^-). As the concentration of the test sample increases, the radical clearing ability of LBP increases gradually.

Result of clearing effect of LBP on superoxide anion (O_2^+)

The result shows that LBP of different concentrations has certain clearing effect on superoxide anion O_2^+, and the clearing effect presents dose-effect linear relationship as the dose increases (Table 4).

DISCUSSION

In this investigation of extraction technology, the extraction method involves cold maceration, without heating, and the main consideration is that polysaccharide is changed easily in high-temperature extraction process. This affects its yield and thereby affects the accuracy of

Table 2. Orthogonal experiment results for LBP extraction technology.

Experiment no.	Factor 1	Factor 2	Factor 3	LBP extraction yield (%)
	Extraction time (h) A	Extraction times B	Solvent volume (ml/g) C	
1	1	1	1	12.56
2	1	2	2	14.85
3	1	3	3	15.21
4	2	1	2	16.32
5	2	2	3	17.54
6	2	3	1	15.92
7	3	1	3	18.66
8	3	2	1	14.59
9	3	3	2	17.67
K_1	18.65	19.61	17.94	-
K_2	20.83	20.73	19.58	-
K_3	21.62	20.77	23.58	-
R	2.97	1.16	5.65	-

Table 3. Experimental result for LBP clearing hydroxy radical (OH^-).

Test sample	Concentration (mg/L)	OH^- clearance rate (%)
1	200	18.5
2	400	45.7
3	600	68.3

Table 4. Result of clearing effect of LBP on superoxide anion O_2^+.

Test sample	Concentration (mg/L)	O_2^+ clearance rate (%)
1	200	15.36
2	400	48.69
3	600	62.51

the results. In addition, some pigments in barbary wolfberry dissolve at high temperature and react easily with polysaccharides. This does not only affect the polysaccharide technology results, but can also bring hidden troubles to subsequent polysaccharide antioxidation experiments. Free radicals are groups or molecules with unpaired electrons and generally have short life, are unstable, active in nature etc.. Under normal circumstances, the human body produces appropriate amount of free radicals for maintaining normal operation of the body. However, as age increases, free radical metabolism in tissues and body fluids slows down, excessive free radicals remain in the body, and the damage caused by free radical reactions leads to human aging and diseases.

Currently, synthetic antioxidants can inhibit oxygen radical reactions, but excessive oxygen radicals in the body can cause bio-membrane damage, protein denaturation, enzyme inactivation and deoxyribonucleic acid (DNA) replication error, thus causing various diseases in the body (Valavanidis et al., 2004; Pincemail et al., 2002). Valavanidis et al. (2004); Olorunnisola et al. (2012) and Saalu et al. (2012) found that cancer (Srivastava and Mittal, 2005; Kannan, 2006), cardiovascular and cerebrovascular diseases (Sagar et al., 1992; Olfat et al., 2012), acquired immune deficiency syndrome (AIDS) and other diseases are related with oxygen radicals; therefore, the adverse effects caused by oxidation reactions coupled with oxygen radicals are of high concern and have gradually become research hotspot. OH^- is active oxygen with the most active chemical properties, and its life is very short.

In the body, OH^- can react with the surrounding molecules and generate level-2 free radicals, so as to destroy cell structure. Studies (Gus'kov et al., 2000; Jin Yue et al.,

2007; Li, 2007a; b) show that some ingredients in some natural plants can effectively clear free radicals, protect body cells and tissues from destruction, effectively resist diseases and delay senility. This experiment explores the clearing effect of LBP on OH^- through Fenton reaction, and the result shows that different concentrations of LBP can clear OH^- radicals. The principle of pyrogallol autooxidation method is to use O_2^+ scavenger to reduce the absorption peak area of pyrogallol autoxidation product at 325 nm and to use ultraviolet spectrophotometer to measure and indirectly calculate the O_2^+ clearance rate for evaluating the antioxidation activity of the test sample. The experimental result shows that LBP has significant effects in clearing O_2^+ radicals, which is consistent with the studies made by other scholars (Meng et al., 2009). This experiment extracts, separates and purifies LBP, to prove the antioxidation activity of LBP.

ACKNOWLEDGEMENTS

This project was supported by 2009 National Supporting Project of Sciences and Technology (No.2009BAI72B03), 2011 National Natural Science Foundation of China (No.81102893) and 2011 Ningxia Science and Technology key projects (No.2011—25)

REFERENCES

Valavanidis A, Nisiotou C, Papageorgiou Y, Kremli I, Satravelas N, Zinieris N, Zygalaki H (2004). Comparison of the radical scavenging potential of polar and lipidic fractions of olive oil and other vegetable oils under normal conditions and after thermal treatment. J. Agric. Food Chem. 52:2358-2365.

Dai W, Li Z, Hu Y (2010). Experimental study on the effects of barbary wolfberry on antioxidation activity in elderly mouse. Pract. Prev. Med. 17(5):874-876.

Gus'kov EP, Varduni TV, Shkurat TP, Milyutina NP, Mirzoyan AV (2000). Free Radicals and Chromosome Aberrations in Leaves of Woody Plants as a test system for the Genotoxicity of the Urban Environment. Russian J. Ecol. 31(4):270-275.

He J, Yan C, Liang Y (1997). Study on chemical composition of barbary wolfberry fruit. Chin. Wild Plant Res. 1(1):8-11.

Huang L, Lin Y, Tian G, Ji G (1998). Study on separation, purification and physicochemical properties of immune active ingredients in barbary wolfberry. Acta Pharm. Sin. 33(7):512-516.

Jiangsu New Medical College (1977). Dictionary of Chinese Traditional Drugs Shanghai: Shanghai Science and Technology Press. 1:1518.

Jin Y, Lv Y, Han G, Sun H, Yu H, Jin F (2007). Comparative study on antioxidation activity of quercetin, isoquercetin and rutin. Chin. Tradit. Herbal Drugs 38(3):408-412.

Pincemail J, Bonjean K, Cayeux K, Defraigne JO (2002). Mécanismes physiologiques de la défense antioxydante physiological action of antioxidant defences. Nutrition Clinique Métabolisme 16:233-239.

Li XM, Ma YL, Liu XJ (2007). Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. J. Ethnopharmacol. 11(3):504-511.

Li XM, Li XL, Zhou AG (2007). Evaluation of antioxidant activity of the polysaccharides extracted from *Lycium barbarum* fruits *in vitro*. Eur. Polym. J. 43(2):488-497.

Meng L, Qiu S, Lan T, Jiang C (2009). LBP ultrasonic extraction technology optimization and antioxidation activity study. J. Anhui Agric. Sci. 37(25):12168-12170.

Olorunnisola OS, Bradley G, Afolayan AJ (2012). Effect of methanolic extract of *Tulbaghia violacea* rhizomes on antioxidant enzymes and lipid profile in normal rats. Afr. J. Pharm. Pharmacol. 6(14):1026 - 1030.

Olfat AK, Kholoud SR, Enas ND, Hanan SA, Najla OA (2012). Antidiabetic activity of *Rosmarinus officinalis* and its relationship with the antioxidant property. Afr. J. Pharm. Pharmacol. 6(14):1031-1036.

Srivastava DSL, Mittal RD (2005). Free Radical Injury and Antioxidant Status in Patients with Benign Prostate Hyperplasia and Prostate Cancer. Indian J. Clin. Biochem. 20(2):162-165.

Saalu LC, Oyewopo AO, Enye LA, Ogunlade B, Akunna GG, Bello J, Ogunmodede OS (2012).The hepato-rejuvinative and hepato-toxic capabilities of *Citrus paradisi* Macfad fruit juice in *Rattus Norvegicus*, Afr. J. Pharm. Pharmacol. 6(14):1056- 1063.

Sagar S, Kallo IJ, Nahni K, Ganguly NK Sharma BK (1992). Oxygen free radicals in essential hypertension. J. Mol. Cell. Biochem. 111(1-2):103-108.

Kannan S (2006). Free radical theory of autoimmunity. J. Theory Biol. Med. Model. 3(22): 1-22.

Wang J, Hu Y, Wang D, Liu J, Zhang J, Abula S, Zhao B, Ruan S (2010). Sulfated modification can enhance the immune-enhancing activity of *Lycium barbarum* polysaccharides. Cell Immunol. 263(2):219-223.

Zhang L, Wang J, Jiang C (2004). Spectrophotometric study on antioxidation activity of active ingredients in Chinese herbs extracted by supercritical fluid CO2. Spectrosc. Spectral Anal. 24(9):1103-1105.