International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

International Journal of Pharmacology 11 (5): 463-469, 2015
ISSN 1811-7775

ansinet
Asian Network for Scientific Information

RESEARCH ARTICLE OPEN ACCESS

DOI: 10.3923/ijp.2015.463.469

Immunoenhancing Constituents of *Poria cocos*

[1]Xiao Hang, [1]Wang Xiaofei, [2]Yang Bingyou, [1]Dou Deqiang and [2]Kuang Haixue
[1]Liaoning University of Traditional Chinese Medicine, 77 Life One Road, DD Port, Dalian, 116600, China
[2]Heilongjiang University of Chinese Medicine, Harbin, China

ARTICLE INFO
Article History:
Received: February 10, 2015
Accepted: April 24, 2015

Corresponding Authors:
[1]Dou Deqiang and [2]Kuang Haixue,
[1]Liaoning University of Traditional Chinese Medicine,
77 Life One Road, DD Port,
Dalian, 116600, China
[2]Heilongjiang University of Chinese Medicine, Harbin, China

ABSTRACT
The dried sclerotium of *Poria cocos* (Schw.) Wolf, referred to as Poria (Fuling in Chinese), was widely used as both food and traditional Chinese medicine. The present study aimed to investigate the immunoenhancing constituents of Poria, multi-mode separation methods that were applied to fraction splitting of Fuling to furnish Petroleum Ether Fraction (PEF), Ethyl Acetate Fraction (EAF), Water Eluated Fraction (WEF), Alcohol Eluated Fraction (AEF) and Crude Polysaccharide Fraction (CPF). The serum hemolysin against SRBC, the Clearance Rate of Carbon Particles, thymus index and Peripheral Blood Lymphocyte (PBL) transformation assays were used to explore the immunoenhancing fractions of Fuling. The minimal effective dose of Poria Water Decoction (WD) for its immunoenhancing action was examined to be 25 g kg^{-1} according to crude drug. Pharmacological experiment indicated that EAF and CPF were regarded as immunoenhancing fractions of Poria, further, the HPLC analysis indicated that triterpenoids are the main constituents of EAF. In addition, the triterpenoids and polysaccharides in different parts of *P. cocos* were compared, indicating the triterpenoids were increased in the order of Poria epidermis (Fuling-Pi)>red Poria (chi-Fuling)>white Poria (bai-Fuling) and the total saccharides and soluble saccharides of red Poria were higher than other parts. The polysaccharides and triterpenoids were the immunoenhancing fractions of Poria and the formere is stronger. The current results can also explain the application difference of the three parts of Poria.

Key words: *Poria cocos* (Schw.) Wolf, polysaccharide, immunopotential material basis, triterpenoids, fuling-Pi

INTRODUCTION

The dried sclerotium of *Poria cocos* (Schw.) Wolf, referred to as Poria (Fuling in Chinese), was widely used as both food and traditional Chinese medicine for more than two thousand years (Jang *et al*., 2011). There are 3 crude drugs derived from Poria, i.e., white Poria (bai-Fuling), red Poria (chi-Fuling) and Poria epidermis (Fuling-Pi) which were named as the characteristic part from the inside to the outside of the sclerotium. The white dense part in the center of its sclerotium was called white Poria. The red porous part close to the epidermis of its sclerotium was called red Poria. The outside layer epidermis of its sclerotium with brown skin was called Poria epidermis. Clinically white Poria and red Poria were always used together which was called Poria with function of spleen invigorating and diuresis. Nevertheless, the Poria epidermis was mainly exhibited the diuretic function and Poria and Poria epidermis were different medicinal materials recorded separately in Chinese pharmacopoeia.

The main action difference between Poria and Poria epidermis is spleen invigorating and immunoenhancement is one of the characteristics of tonifying spleen according to the theory of traditional Chinese medicine. A research indicated that Poria polysaccharides possessed immunoenhancing action (Chen and Chang, 2004), however, most of the pharmacological evaluation is just based on the individual fractions or individual compounds which could not reflect the real representative constituents of the drug. In this study, firstly the minimal effective dose of Fuling Water Decoction (WD) explored was and then the doses of the splitted fractions

were determined with corresponding recovery rates. Thus, the real immunoenhancing constituents of Fuling can be elucidated. Previously, the splitting method for chemical fractions of Fuling was established and the chemical constituents of splitted fractions of Fuling were identified. In addition, the distinctive degrees among the fractions were firstly analyzed to evaluate their overlapping property of splitted fractions of Fuling and the diuretic fraction of Poria was also examined (Lin *et al*., 2013; Li *et al*., 2015). This study aimed at evaluating the effects of different chemical fractions of Poria on immunoenhancing action through specific and nonspecific immune pharmacological experiments to elucidate its chemical basis for spleen replenish action.

MATERIALS AND METHODS

Chemicals and reagents: Agilent 1260 series HPLC was purchased from Agilent Technologies, Inc. (USA). Chromatographic C-18 column (250×4.6 mm, 5 μm) was made from Kromasil Corporation (Sweden). UV-2100 spectrophotometer was purchased from Unico Co. (Shanghai, China). Freeze-drying machine was purchased from Labconco Corporation (England). Petroleum ether (analytical grade) was obtained from Jinfen Chemicals Ltd (Tianjin, China); methanol (HPLC grade) was obtained from Kermel Chemicals Ltd (Tianjin, China); acetonitrile was obtained from Kermel Chemicals Ltd (Tianjin, China); normal saline was obtained from Kelun Pharmaceutical Ltd (Heilongjiang, China). PHA was obtained from Zhurui Bio-Technique Co. Ltd (Shanghai, China). Indian ink was obtained from Solarbio Ltd (Beijing, China). Other reagents were all of analytical grade.

Animals: Animals (Healthy KM mice, weighed 20.0±2 g, male) for experiment were purchased from laboratory animal center of Changsheng Bio-Technique Co. Ltd, qualified number SCXK 2010-0001 (Benxi, Liaoning, China). The animals were housed in an air-conditioned room (temperature, 25°C; relative humidity, 55%) and fed *ad libitum* with standard feed and water in the course of the study. At the end of experiment, pentobarbital sodium was used for euthanasia of the animals. The investigation conforms to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH Publication No. 85-23, revised 1996). The study protocol was approved by the ethics regulations of Liaoning University of TCM (131/2010).

Plant materials: The commodities of Fuling were purchased from Tongrentang Pharmaceutical Ltd (Dalian, China) and the material of Fuling was collected from Yunnan, China, in the November of 2012 and all the samples were identified as sclerotium of *Poria cocos* (Schw.) Wolf by Prof. Bing Wang, College of Pharmacy, Liaoning University of Traditional Chinese Medicine. A voucher specimen (Batch No. 20121101) has been deposited at the College of Pharmacy, Liaoning University of Traditional Chinese Medicine.

Fraction splitting of poria: The fraction splitting procedure of Poria was performed according to reference (Li *et al*., 2015) and 5 fractions were furnished. By the isolation and identification, the chemical constituents of the five fractions were elucidated. It can be concluded that PEF mainly contains aromatic esters, EAF mainly contains triterpenoids, WEF mainly contains small molecular saccharides, AEF mainly contains amino acid and indole compounds, CPF mainly contains crude polysaccharides.

Determination of serum hemolysin against sheep red blood cell (SRBC)

Preparation of sheep red blood cell: The blood was taken from sheep jugular vein and placed in a sterile conical flask with beadings, shaking clockwise to eliminate fibre for 15 min. Appropriate normal saline was added into flask and centrifuged at 2000 rpm for 10 min to wash the red blood cell until the supernatant layer was clarified. Discarded the supernatant solution to yield the red blood cell and was kept in 4°C.

Test of serum hemolysin against sheep red blood cell (SRBC): The assay was performed according to reference with minor modification (Liu *et al*., 2007). The lowest effective dose of Fuling water decoction for promoting serum hemolysin against SRBC was determined to 25 g kg^{-1} raw herbs. According to recovery rates of Fuling separation fractions, the doses of these separated fractions were determined. The experimental animals were divided into 7 groups randomly. Every morning at 8:00 am, the 6 groups of Kunming mice were administered the 5 separated fractions and Fuling water decoctum, respectively which were dissolved in 1% tween-80. Blank group was administered 0.2 mL/10 g 1% tween-80. On the third day of administration, the animals were sensitized with 0.2 mL 5% SRBC. On the seventh day, blood was taken from retroocular venousplexus of the mice after 1 h of administration. Serum was separated by centrifugation at 3000 rpm for 15 min and 1 mL serum was added and diluted 1000-fold by normal saline, 0.5 mL 5% SRBC diluted 20-fold by normal saline and 1 mL 10% serum complements diluted 10-fold by normal saline into 15 mL graduated centrifuge tubes, then keep the tubes at 37°C for 30 min, terminate the reaction for 5 min in ice-bath, centrifuge these tubes at 1500 rpm for 10 min. The absorbance of the supernatant was detected with UV-2100 at 540 nm.

Determination of the indexes of the clearance rate of carbon particles: The assay was performed according to reference with modification (Pei *et al*., 1993; Ding *et al*., 1994). The lowest effective dose of Fuling water decoction for raising clearance rate of carbon particles of Kunming mice administered 9 days was investigated to be 25 g kg^{-1} raw herbs. According to recovery rates of Fuling separation fractions, the doses of these separated component groups were determined. The 70 healthy Kunming mice were divided into

7 groups randomly, the 6 groups of Kunming mice were administered respectively with the separated fractions which were dissolved in 1% tween-80. Blank group was administered 0.2 mL/10 g 1% tween-80. After 1 h of the seventh day administration, weigh the Kunming mice, then blood was taken from retro-ocular venous plexus of the mice at precise time of 2 and 10 min, with initiating the time of intravenous injection of Indian ink diluted 4-fold by normal saline. About 20 μL blood was taken in time before blood clotted, then added the blood to 2 mL 0.1% Na_2CO_3, mixed. The absorbance of the mixture was detected with UV-2100 at 680 nm. Mice were executed to get integrated tissues of spleens, livers and thymus. After their fluid and blood was dried by filter, the organs were weighed. The formulas of K value for clearance of carbon particles, α value for the macrophage phagocytic index and organ index were calculated as follows:

$$K = \frac{LogOD_2 - \log OD_{10}}{T_{10} - T_2} = \frac{LogOD / OD_{10}}{8}$$

$$\text{Organ index} = \frac{\text{Organ weight}}{\text{Body weight}}$$

$$\alpha = \frac{\text{Body weight}}{\text{Organ weight}} \times K^{1/3}$$

Determination of peripheral blood lymphocyte (PBL) transformation: The lowest effective dose of Fuling water decoction for raising PBL transformation of Kunming mice with 9 days administration was investigated to be 25 g kg^{-1} raw herbs. According to recovery rates of Fuling splitted fractions, the doses of these separated fractions were determined. From the first day, Kunming mice received intramuscular injection of PHA for consecutive 3 day. On the second day, every morning at 8:00, the 6 groups of Kunming mice were administered the separated fractions respectively which were dissolved in 1% tween-80. Blank group was administered 0.2 mL/10 g 1% tween-80. From the fifth day, observe the number of lymphoblast and transitional cells per 100 lymphocytes and calculate the PBL transformation rate (Xu, 1982).

Determination of triterpenoids of fuling by HPLC

Preparation of standard solutions for content analysis: Pachymic acid, dehydropachymic acid, 3-O-acetyl-16-hydroxytrametenolic acid were taken as standards and dissolved with HPLC grade methanol at the concentration of 1.036, 1.455 and 1.08 mg mL^{-1}, respectively. All samples were filtered through 0.45 μm before analysis.

Preparation of sample solution: The dried sclerotium of Fuling was cut and divided into four parts from outside to inside and marked as A, B, C and D, respectively and the D part is consisted mainly of Fuling-Pi. All the samples were

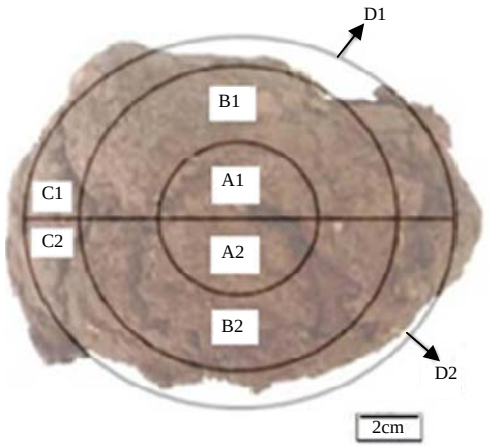

Fig. 1: Cutting modes of fuling

pulverized to pass through 60 mesh sieve and predried to constant weight at 50°C 2.5 g of Fuling samples were accurately weighed and ultrasonic-extracted with 25 mL methanol for 30 min (Fig. 1). The extract solutions were filtered and constant to 5 mL and filtered through 0.45 μm membrane before HPLC analysis.

Chromatographic condition: Analysis of the 3 individual triterpenoids was performed in an Agilent 1260 series HPLC system with a ZORBAX SB-C18 (250×4.6 mm, 5 μm) protected by a pre-column from the same company and equipped with DAD detector, eluted with acetonitrile (A) and water-phosphoric acid (100:0.5, B) in gradient at the flow rate of 1 mL min^{-1}. The solvent gradient for determining consisted of 25% A at the beginning, 50% A at 10 min, 60% A at 20 min, 70% A at 40 min, 75% A at 55 min column temperature was thermostated at 35°C. The HPLC chromatograms were determined at 242 and 210 nm. The injection volume was 10 μL for each sample. Peak identification was performed by comparison of their retention times.

Validation of HPLC method: The method developed above was validated for linearity, precision, repeatability, stability and recovery. Linearity was evaluated based on linear regression analyses of the three components. The peak areas for the same concentration were averaged. Using this data a linear regression model was developed and a regression coefficient greater than 0.999 was considered to have a linear relationship between the peak area and the concentration of the analyte. The equations of pachymic acid, dehydropachymic acid and 3-O-acetyl-16-hydroxytrametenolic acid were determined as: y = 7503x-48.40 (r = 0.9995), y = 12495x+14.52 (r = 0.9995) and y = 3980x-18.45 (r = 0.9995), respectively. Precision was assessed with the reference substance solution in a certain content for six times

and repeatability was assessed with six times of the same Fuling sample. The identical sample was injected continuously after 0, 1, 2, 6, 12, 24 h to evaluate the stability of the method. The Relative Standard Deviation (RSD) was used to evaluate the precision, repeatability and stability which showed that the RSD were all less than 3%. Recovery analysis was performed with six batches of Fuling, revealing the percentages for pachymic acid (100.01%±0.23), dehydropachymic acid (99.70%±1.12) and 3-O-acetyl-16-hydroxytrametenolic acid (98.34%±2.73).

Determination of saccharides in fuling

Determination of total polysaccharides: Glucose (20 mg) was diluted to 100 mL with distilled water to make standard solution. Saccharides content of different parts were determined by phenol-sulfate method (Xu, 1982) as the equation of glucose as:

$$y = 0.014x-0.008 \ (r = 0.9995)$$

where, x is the concentration of glucose and y is the absorbance. The 50 mg of Fuling powder predried to constant weight at 50°C was weighted accurately and 15 mL HCl (1 mol L^{-1}) was added into samples and hydrolyzed for 2 h in boiling water bath. NaOH solution (10%) was added to neutralize until neutral. After cooling, then filtered and add distilled water to the volume of 50 mL. About 0.2 mL of sample solution was taken into 1.8 mL of water, then mixed with 1 mL of phenol (6%) and then, 5 mL of concentrated sulfuric acid was added slowly and reacted for 20 min in the boiling water bath. The absorbance value was measured spectrophotometrically in the ultraviolet reader at a wavelength of 490 nm. The content was calculated by the external standard two-point method according to glucose curve.

Determination of soluble polysaccharide content: The 0.5 g of Fuling powder were accurately weighed and extracted with 50 mL 80% ethanol, then centrifuged at 4000 rpm for 15 min and discarded the supernatant and then, repeated this process for 2 times. After drying the residue, 25 mL distilled water was used to extract the residue for 2 h in boiling water bath. The resulting water extract was evaporated in vaccum to 10 mL and 95% ethanol was added until the ethanol concentration up to 80%, standstill for 12 h at 4°C. Centrifuged and the precipitate was dissolved in 5 mL distilled water, the soluble polysaccharide content was determined by phenol-sulfate method as reference (Yang and Li, 2007).

RESULTS

Test of serum hemolysin content against sheep red blood cell: Serum hemolysin against Sheep Red Blood Cells (SRBC) was the antibody level which was secreted from B lymphocytes. The degree of hemolytic reaction when antibody incubated with SRBC *in vitro* reflected the level of antibody and was assayed by the method of spectrophotometry. The results indicated that EAF and CPF groups could increase the value of serum hemolysin and of serum hemolysin and antibody level significantly compared with the control group (Table 1), revealing that the main effective separated components were EAF group and CPF group.

Table 1: Effects of serum hemolysin against sheep red blood cell (x±S, n = 10)

Groups	Value of serum hemolysin
Control	0.63±0.27
WD	1.12±0.50*
PEF	0.56±0.35
EAF	1.16±0.56*
CPF	1.19±0.57*
WEF	0.27±0.21
AEF	0.62±0.28

* $0.01<p<0.05$ compared with control group, WD: Water decoction, PEF: Petroleum ether fraction, EAF: Ethyl acetate fraction, CPF: Crude polysaccharide fraction, WEF: Water eluated fraction, AEF: Alcohol eluated fraction

Effects of indexes of the clearance rate of carbon particles: The phagocytic ability of mononuclear macrophage was one of the main indicators which reflected the function of nonspecific immunity. The elimination rate was exponential with the blood carbon concentration in a certain range. In addition, spleen and thymus were important immune organs which could secrete cytokines to affect the activity of macrophages. The indexes of spleen and thymus reflected the function of organs. The present results showed that all the fractions of Fuling can increase the thymus index, revealing that all the components of Fuling had a certain influence on the phagocytosis of macrophage and the PEF group, EAF group and CPF group can increase the spleen index significantly compared with control group (Table 2). Phagocytic index was calculated with K value and weigh of thymus and liver. The phagocytic index of CPF group was significantly higher than the control group, revealed that crude polysaccharide fraction may be the main active composition in enhancing the immune system.

Effects of Peripheral Blood Lymphocyte (PBL) transformation: The level of lymphocyte transformation can reflect the level of cellular immunity. The PBL transformation rate of T lymphocytes stimulated by PHA was elevated to the highest level on the sixth day of the final intramuscular injection with PHA. Compared with control group, there was no significant difference in PEF group, WEF group and AEF group from the third day to the seventh day of the final intramuscular injection with PHA. The PBL transformation rates of EAF group and CPF group from the third day to the sixth day of the final intramuscular injection with PHA were observed to be significant difference as compared with the control group but there was no difference in the seventh day of the final intramuscular injection with PHA (Fig. 2). The results revealed that polysaccharides constituent and triterpenoids were the active composition of Poria for the PBL transformation.

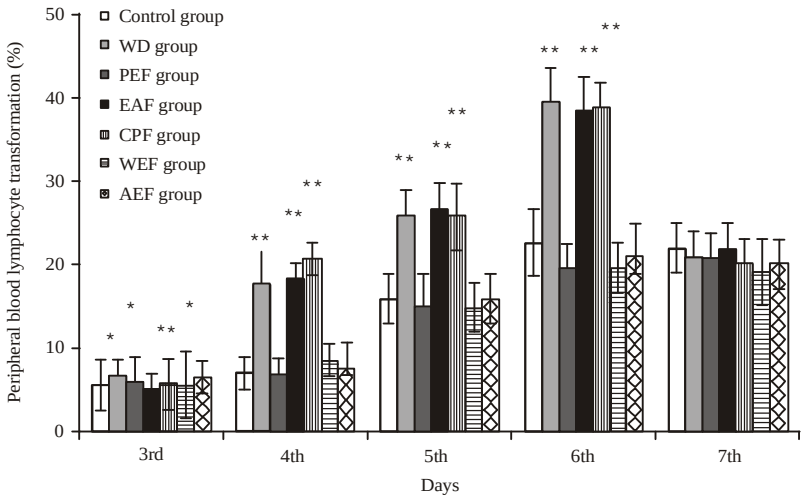

Fig. 2: Effects of Peripheral Blood Lymphocyte (PBL) transformation (*p<0.05, **p<0.01 compared with control group)

Table 2: Effects of the indexes of the clearance rate of carbon particles (x±SD, n = 10)

Groups	Thymus index (mg g^{-1})	Spleen index (mg g^{-1})	Carbon clearance index K(×100)	Phagocytic index α
Control	0.532±0.31	2.316±0.62	0.755±0.29	4.35±0.60
WD	2.175±0.44**	2.980±0.40*	1.048±0.24*	4.89±0.50*
PEF	2.107±0.53**	3.064±0.56*	0.812±0.19	4.46±0.38
EAF	2.720±0.53**	3.000±0.69*	0.863±0.15	4.56±0.34
CPF	1.891±0.56**	3.020±0.34*	1.072±0.30*	5.41±0.64**
WEF	2.143±0.31**	2.960±0.64*	0.897±0.25	4.90±0.51*
AEF	2.897±0.66**	2.630±0.43	0.820±0.21	4.56±0.51

*p<0.05,**p<0.01 compared with control group, WD: Water decoction, PEF: Petroleum ether fraction, EAF: Ethyl acetate fraction, CPF: Crude polysaccharide fraction, WEF: Water eluated fraction, AEF: Alcohol eluated fraction

Table 3: Triterpenoids and polysaccharides of Poria (mg g^{-1}, x±SD, n = 3)

Groups	Medicine part	PA	DA	TA	TS	SS
Commerce	White poria	0.5797±0.010	0.2611±0.004	0.2990±0.014	372.9±3.2	1.868±0.04
Products	Red poria	0.9136±0.010	0.5186±0.001	0.7941±0.006	612.1±4.3	4.237±0.08
	Poria epidermis	1.3342±0.088	0.8814±0.094	1.7918±0.003	388.8±1.3	1.592±0.05
Poria	A	0.4315±0.006	0.2784±0.004	0.3052±0.024	382.1±5.1	1.790±0.04
	B	0.4661±0.003	0.3105±0.003	0.3631±0.019	347.0±4.2	2.020±0.05
	C	0.5206±0.028	0.3334±0.007	0.3968±0.014	430.1±5.7	3.420±0.05
	D	0.7517±0.018	0.4950±0.007	0.7657±0.033	387.0±3.7	1.520±0.05

Note: PA: Pachymic acid, DA: Dehydropachymic acid, TA: 3-O-acetyl-16-hydroxytrametenolic acid, TS: Total saccharides, SS: Soluble saccharides

Triterpenoids in different commodities and different parts of Poria: Pachymic Acid (PA), Dehydropachymic Acid (DA) and 3-O-acetyl-16-hydroxytrametenolic acid (TA) were chosen as indexes to compare the difference. The results showed that the triterpenoids in different commercial products of Poria were significantly different with the contents of Poria epidermis>red Poria>white Poria. Based on the origin of Poria and Poria epidermis from the dried sclerotium of Poria, it can be generally considered that part D is for the Poria epidermis, part C is mainly composed of red Poria and part B is white Poria. The results showed that the content of pachymic acid, dehydropachymic acid and 3-O-acetyl-16-hydroxytrametenolic acid in D were significantly higher than other parts. The contents of triterpenoids were increased from inside to outside of whole Poria crude materials as in the order of D>C>B>A (Table 3, Fig. 3). Based on the two parts results, it can generally concluded that the triterpenoids contents in different parts of Poria is Poria epidermis>red Poria>white Poria.

Polysaccharides content of Poria: Polysaccharides were determined by phenol-sulfate method and the results indicated that the polysaccharides of red Poria is higher than white Poria and Poria epidermis. In addition, the content of the soluble polysaccharides in part C showed higher than other parts (Table 3). Thus, red Poria possessed higher polysaccharides but the contents of polysaccharides in other parts exhibited the same level.

DISCUSSION

Immunoenhancing action is associated with the spleen tonifying action the perspective of traditional Chinese medicine (Chen *et al.*, 2010). Immune system was consisted of immune organs, immune cells and immune molecules and can be classified as nonspecific immunity and specific immunity. Nonspecific immunity was also called innate immunity which human gained by nature and it included

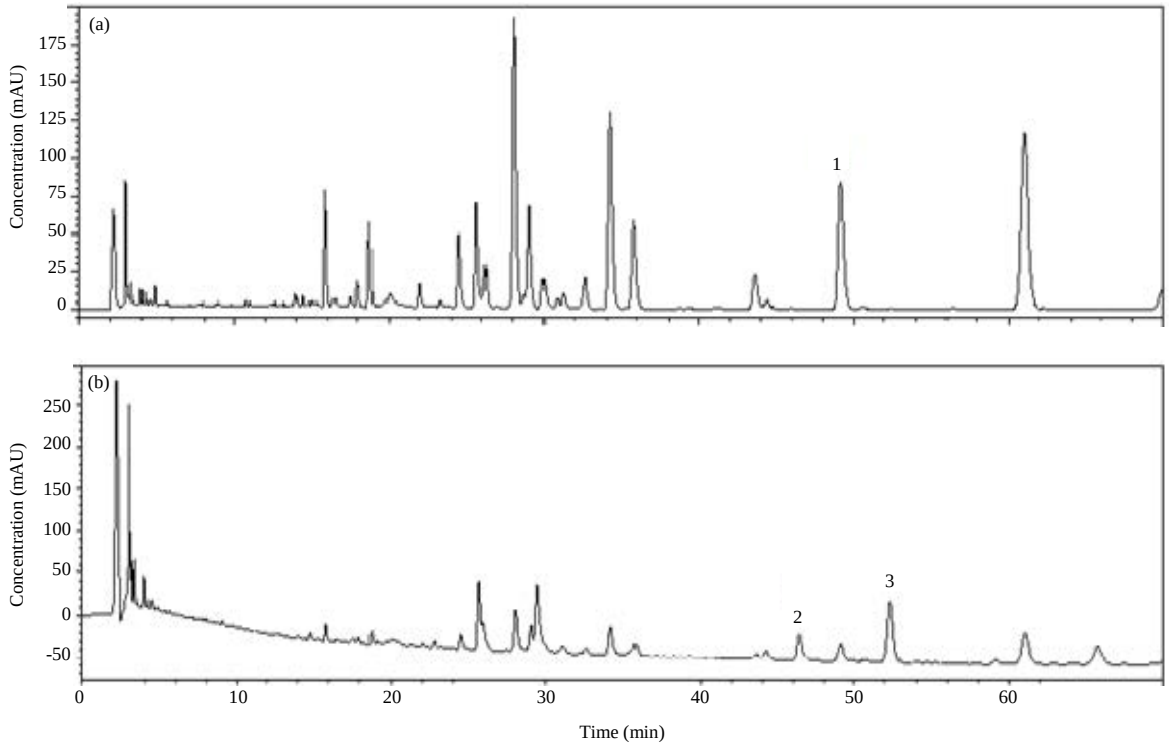

Fig. 3(a-b): HPLC chromatogram of part C of Fuling at (a) 242 nm and (b) 210 nm, (PA) 1: Pachymic acid, 2: Dehydropachymic Acid (DA), 3: 3-O-acetyl-16α-hydroxytrametenolic acid (TA))

physiological barrier, nonspecific immunocyte and immune molecules, among which the mononuclear phagocyte played an important role in immune function (Strauss *et al*., 2015). The phagocytic function of mononuclear phagocyte reflected the ability of nonspecific immune system. Specific immunity was also called acquired immunity and generally formed when organism stimulated by an antigenic substance which concluded humoral immunity and cellular immunity. In present experiments, the serum hemolysin against sheep red blood cell can reflected the situation of humoral immunity. The present results revealed that the main effective splitted fractions were EAF and CPF. The phagocytic ability of mononuclear macrophage was one of the main indicators which could reflect the function of nonspecific immunity (Strauss *et al*., 2015). The phagocytic ability of reticuloendothelial cells was evaluated by i.v. Indian ink. The elimination rate was exponential relationship with the blood carbon concentration in a certain range. In addition, spleen and thymus were important immune organs which could secrete cytokines to affect the activity of macrophages. The present results showed that the PEF, EAF and CPF groups can increase the spleen index significantly as compared with control group. The phagocytic index of CPF was significantly higher than the control group, revealed that saccharides may be the main active compositions for the innate immunity. The transformation level of lymphocyte could reflect the ability of immune system and was one of the major indexes in evaluating the cytoimmunity (Alipour *et al*., 2014). The results showed that the PBL transformation rates of EAF group and CPF group from the third day to the sixth day of the final intramuscular injection with PHA, exceeded that of control group, revealing that saccharides and triterpenoids of Poria are the active components. Consequently, the polysaccharides and triterpenoids were the immunoenhancing fractions of Poria and the formere is stronger.

In addition, the triterpenoids and saccharides in different parts of Poria were compared. Content analysis was not recorded in both Poria and Poria epidermis in China's pharmacopoeia (2010 edition). The present study revealed that the triterpenoids in Poria epidermis is the highest but the soluble polysaccharides is the least. Red Poria contained the highest total polysaccharides and soluble polysaccharides and white Poria contained the lowest triterpenoids but its contents were closed to red Fuling. China's pharmacopoeia, Poria and Poria epidermis have recorded separately with different functions. The crude drugs of Poria, originated from red Poria and white Poria together, possessed the functions of diuretic and spleen replenishing action but the Poria epidermis mainly used as a diuretic drug. The previous research indicated that the EAF is the diuretic fractions of Poria (Li *et al*., 2015). The present pharmacological and analytical results further disclosed that the application difference of Poria and Poria

epidermis in traditional Chinese medicine is reasonable. It is for the first time that we found EF mainly composed of triterpenoids possessed the immunoenhancing action which provide an evidence for the various usage of Poria and Poria epidermis in both food and medicine. In TCM, the function of spleen is also associated with digestion and absorption of foods, the pharmacological effects of Poria on this aspect are still in progress.

ACKNOWLEDGMENT

The research project was supported by grants from National Basic Research Program of China (2013CB531803) and Program for Liaoning Innovate Research Team in University (LT2013020).

REFERENCES

Alipour, R., M. Adib, B. Hashemi-Beni and F. Sadeghi, 2014. The effect of stem cell from human exfoliated deciduous teeth on T lymphocyte proliferation. Adv. Biomed. Res. Sci., Vol. 3. 10.4103/2277-9175.142312

Chen, X.X., Y.J. Zhang, M.X. Li and Y. Wu, 2010. The research of the relationship of spleen deficiency and immune system. China Mod. Med., 34: 6-8.

Chen, Y.Y. and H.M. Chang, 2004. Antiproliferative and differentiating effects of polysaccharide fraction from fu-ling (*Poria cocos*) on human leukemic U937 and HL-60 cells. Food Chem. Toxicol. Sci., 42: 759-769.

Ding, Y., P. Chen, S. Xing and X.B. Ru, 1994. Effect of *Epimedium polysaccharide* against inhibitory action of azidothymidine on mouse hemopoietic and immune functions. Chin. J. Pharmacol. Toxicol., 8: 203-205.

Jang, T.R., M.F. Kao, C.H. Chen, K.C. Hsieh, W.Y. Lai and Y.Y. Chen, 2011. Alleviating effects of dehydration under no hyperthermia on the immunomodulatory response to the polysaccharide fraction from fu-ling (*Poria cocos*) in male collegiate wrestlers. Chin. Med. J. Sci., 124: 530-536.

Li, B., Y.X. Ding, D.Q. Dou, X.K. Ran, Y.B. Xu, L.H. Li and T.G. Kang, 2015. Diuretic ingredients of *Poria coco*. Int. J. Pharmacol.

Lin, Z., Y.B. Xu, X.K. Ran and D.Q. Dou, 2013. [Splitted fractions and unoverlapping analysis of chemical constituents of *Poria cocos*]. China J. Chin. Materia Medica, 38: 4340-4346, (In Chinese).

Liu, J., M. Gao, H. Yu, Y. Wang, K. Wu, H. Yuan and C. Zhu, 2007. Efects of wild lactarius volemus from Guizhou province on immunological function in mice. Chin. J. Public Health, 23: 291-292.

Pei, R.J., L.H. Qi and X.J. Liu, 1993. Effects of triptonide on mouse immune functions. Acta Pharmacol. Sin., 14: 238-242.

Strauss, O., P.R. Dunbar, A. Bartlett and A. Phillips, 2015. The immunophenotype of antigen presenting cells of the mononuclear phagocyte system in normal human liver: A systematic review. J. Hepatol., 62: 458-468.

Xu, S.Y., 1982. Methodology of Pharmacological Experiment. People's Medical Publishing House, Beijing, China, Pages: 952.

Yang, W.D. and G.G. Li, 2007. Content analysis on total sugar and polysaccharose in *Poria* student prouduct and other different processing products. Chin. J. Hosp. Pharm., 7: 916-918.