REPORT

In vitro immune potentials of a water-soluble polysaccharide extract from *Dioscorea opposita* planted in Henan Province, China

Li-Xin Hao[1] and Xin-Huai Zhao[1,2]

[1]Key Laboratory of Dairy Science, Ministry of Education, Northeast Agricultural University, Harbin, PR China

[2]Department of Food Science, Northeast Agricultural University, Harbin, PR China

Abstract: *Dioscorea opposita* is an edible and medicinal plant available in many areas of China. This study aimed to assess *in vitro* immune potentials of a water-soluble polysaccharide extract from *D. opposita* planted in Henan Province, China. *In vitro* effects of the extract on three immune cells (macrophages, natural killer cells and splenocytes) from mice and secretion of eight immune-related molecules in macrophages and splenocytes were evaluated. In total, the extract exhibited a dose-dependent manner on these immunological responses. The extract at dose level of 50μg/ml enhanced respective splenocyte proliferation, macrophage phagocytosis, and natural killer cell activity by 150%, 18% and 47%, increased secretion of interleukin-2 and interferon-γ (from 41.4 and 24.6 pg/ml to 48.8 and 91.5 pg/ml, respectively) but decreased secretion of interleukin-4 (from 38.9 to 27.9 pg/ml) in splenocytes. The extract at the same dose level also stimulated inducible nitric oxide synthase and lysozyme in macrophages, and enhanced secretion of interleukin-6, interleukin-1β and tumor necrosis factor-α (from 26.6, 73.4 and 39.6 pg/ml to 60.2, 131.0 and 144.7 pg/ml, respectively). It is concluded that water-soluble polysaccharides from *D. opposita* have immune potentials to the body, via activating immune cells and regulating the secretion of immune-related molecules.

Keyword: *Dioscorea opposita,* soluble polysaccharides, immune activity, immune cells, immune molecules.

INTRODUCTION

Many natural compounds have a variety of biological activities (Peters, 2006). Among these natural compounds are polysaccharides from plants, animals and microorganisms (Choi *et al*., 2004; Yuan *et al*., 2010). Polysaccharides might have healthcare functions such as antitumor (Saima *et al*., 2000; Hsu *et al*., 2004; Leung *et al*., 2006), anticoagulation (Hussein *et al*., 1998), anti inflammation (Scheppach *et al*., 2004), antialexin (Yamada, 1994), antioxidation (Liu *et al*., 1997) and immunostimulation (Tzianabos *et al*., 2003), and have attract more attention both in biochemical and medical areas (Ooi and Liu, 2000). Immune function, one of these important physiological functions in the body, is extendedly verified for natural polysaccharides. A variety of potential pathogenic microorganisms (viruses, bacteria and others) can infect the cells and multiply, in a few cases, may cause disease (Dunn *et al*., 2004; Raman *et al*., 2006). It is absolutely necessary to maintain the body with normal immune function, as primary function of immune system is to eliminate infectious microbes, viruses and tumors (Sideras *et al*., 2014). Immune system of the body consists of immune organs, immune cells, and immunoactive substances (Calder *et al*., 2006). Lymphocytes and macrophages both are the most important immune cells, while lymphokines, antibody, lysozyme and others are considered as the important immune-related substances. Natural compounds including polysaccharides have thus been assessed for their potentials in the enhancement of both immune cells and immune-related molecules.

Dioscorea opposita is a well-known edible and traditional medicinal plant (Liu *et al*., 1995), and is available in many areas of China. *D. opposita* can be used alone or in multiple-herb formulations to invigorate the stomach and spleen, and is beneficial to lungs and kidneys (Farombi *et al*., 2000). Some compounds isolated from *D. opposita* have both healthcare functions and medicinal application; for example, a cyclic compound allantoin from *D. opposita* tuber can expedite healing process, while diosgenin from *D. opposita* root can be used in the production of a number of medicines (Zava *et al*., 1998). Recent study results show that *D. opposita* has some specific biological effects including anticancer (Saima *et al*., 2000), induction of hypoglycemia (Hsu *et al*., 2007), as well as antibacterial (Hriram *et al*., 2008), antioxidant (Farombi *et al*., 2000), hypolipidemic (Son *et al*., 2007) and immunomodulatory (Zhu *et al*., 2005) activities. Immune activities of a mucopolysaccharide extract from *D. opposita* towards both splenocytes and macrophages have been assessed *in vitro* (Choi *et al*., 2004). However, the mucopolysaccharide extract is only purified by sodium dodecyl sulfate to remove protein fractions. And more, if the mucopolysaccharide extract has effect on the

**Corresponding author:* e-mail: zhaoxh@neau.edu.cn

secretion of those important immune-related molecules in both macrophages and lymphocytes is still not studied. It is necessary to find more scientific evidence about immune activities of *D. opposita* polysaccharides towards the body.

In the present study, a polysaccharide extract was thus prepared by water extraction of *D. opposita* followed by two-step purification (phase separation by Sevage reagent and protease hydrolysis), and then investigated for its *in vitro* immune potentials.

MATERIALS AND METHODS

Materials and chemicals

D. opposita planted in Henan Province, China, was purchased from a local market of Harbin (Heilongjiang Province, China). Typan blue and neutral red both were purchased from Amresco Inc. (Los Angeles, California, USA). Cell Counting Kit-8 (CCK-8) was purchased from Dojindo Laboratories (Dojindo Laboratories, Kyushu, Japan). Concanavalin A (Con A) was purchased from Sigma Aldrich (St. Louis, Missouri, USA). Mouse inducible nitric oxide synthase (iNOS) and lysozyme (LZM) enzyme-linked immunosorbent assay (ELISA) kits were all purchased from Beijing Equation Biotechnology Science and Technology Co. Ltd. (Beijing, China). These used cytokines kits were provided by Wuhan Boster Biological Engineering Co. Ltd. (Wuhan, Hubei, China). All chemicals used of analytical grade.

Animals and cells

Female BALB/c mice were purchased from Beijing Vital River Experimental Animal Technical Co. Ltd. (Beijing, China). All mice were 6 to 8-week-old, and maintained in an animal facility for at least one week before experiment. All animal procedures were performed in accordance with the ethical guidelines of the Animal Care and Use Committee of Northeast Agricultural University (Harbin, Heilongjiang, China).

The YAC-1 cells used for the assay of natural killer (NK) cell activity were purchased from Chinese Academy of Sciences (Shanghai, China).

Preparation of the water-soluble polysaccharide extract

D. opposita was ground and extracted with water in a proportion of 1:10 (w/w) at pH 4.5. The extraction was carried out at 100°C for 3 h. The mixture was cooled to room temperature, and centrifuged at 8,000 r/min for 15 min to collect the resultant supernatant. The supernatant was mixed with the Sevage agent (chloroform: n-butyl alcohol, 5:1, v/v) at room temperature for 5 min, and then centrifuged at 5,000 r/min for 5 min to discard the upper phase. The lower phase was neutralized into pH 7.0, and treated at 55°C for 3 h by an alkaline protease (Alcalase) to digest protein fractions. After heating at 100°C for 5 min to inactivate the protease, the hydrolysates was cooled and centrifuged at 8,000 r/min for 5 min. The collected supernatant was concentrated at 100°C into 1/5 of its original volume. Absolute ethanol was added into the concentrated supernatant, ensuring final ethanol concentration of 75% (v/v). The mixture was kept at 4°C for 24h and centrifuged to separate the precipitated material. The precipitated material was dissolved in water, and precipitated by absolute ethanol again. The final obtained material (water-soluble polysaccharide extract) was lyophilized, and evaluated for saccharide content as per the method of Dubois *et al*. (1951). The extract was dissolved in sterilized water before evaluation of its immune activities, while sterilized water was used as control.

Assay of lymphocyte proliferation

Spleens were aseptically removed from the BALB/c mice, put in the Hank's balanced salt solution, cut into pieces, and passed through a 200-mesh sieve to make single cell suspension. The suspension was centrifuged at 1,000 r/min for 5 min to remove the supernatant, treated by red cell lysis buffer of 5ml for 3 min, and then centrifuged at 1,000r/min for 5min. Viability of the detached splenocytes was assessed using the trypan blue exclusion test to ensure the value >98%. Splenocytes were suspended at a final density of 2×10^5 cells/ml in RPMI-1640 medium and seeded onto a 96-well plate (100μl/well). Serial solutions of the extract of 80μl were added into each well to achieve final saccharide levels of 10, 30 and 50μg/ml, respectively. These dose levels were used again in other evaluation. Con A of 20μl was added into each well to achieve final concentration of 7μg/ml, followed by an incubation at 37°C in 5% CO_2 for 48 h. CCK-8 of 20μl was added into each well, and the cells were incubated for 4 h again. The absorbencies were measured at 450 nm by a micro plate reader (Bio Rad Laboratories, Hercules, CA, USA), and the values (A_{450}) were used to report lymphocyte proliferation.

Assay of phagocytosis of peritoneal macrophages

Peritoneal macrophages were aseptically isolated from the mice injected with 10 ml of the Hank's balanced salt solution. The collected cells were centrifuged at 1,000 r/min for 5 min. Viability of the detached cells was also assessed by the trypan blue exclusion test to ensure the value >98%. The cells were suspended at a final density of 2×10^5 cells/ml in the RPMI-1640 medium, seeded onto a 96-well plate (100μl/well) and cultured at 37°C in 5% CO_2 for 4 h before discarding the suspension. Both RPMI-1640 medium and the extract solutions (100μl/well) were added into each well. The cells were incubated for 24 h before discarding the suspension; after that, neutral red solution (1%, diluted by saline) of 100μl was added into each cell. After further incubation of 30 min, the cells were washed three times by 1mol/L phosphate buffer saline (PBS) to remove excess neutral red. A mixture composing of acetic acid (1%) and ethanol (95%) (1:1, v/v) was added into each well at 100 µl/well, and the cells were incubated again for 2 h. The absorbencies were measured at 540 nm by the micro plate reader, and the values (A_{540}) were used to reflect macrophage phagocytosis.

Assay of NK cell activity

NK cell activity was assayed using a CCK-8 assay kit. Splenocytes (5×10^5 cells/ml) and YAC-1 cells (1×10^4 cells/ml) of 50 µl were plated onto a 96-well plate, and the extract solutions of 50 µl were also added into each well. The cells were incubated at 37°C for 20 h, and then CCK-8 of 15 µl was added into each well. Following another 4 h of co-culture, the absorbencies were measured at 450 nm using the micro plate reader. At the same time, the absorbencies were also recorded for the target and effect or cell controls. The percentage of NK cell activity was calculated as per the method of Yuan *et al*. (2010).

Assays of the levels of eight immune-related molecules

Splenocytes or macrophages (2×10^5 cells/ml) of 2 ml were seeded onto 6-well plates. The extract solutions of 1 ml were added into each well. The cells were cultured at 37°C for 48 h, and then centrifuged at 2,000 r/min for 20 min to collect cellular supernatants. The collected supernatants were then assayed for the levels of eight immune molecules, including interleukin-2 (IL-2), interleukin-4 (IL-4), interferon-γ (IFN-γ), interleukin-6 (IL-6), interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), iNOS and LZM, using the respective kits and protocols provided by the kits manufacturer.

STATISTICAL ANALYSIS

All data were expressed as mean values ± standard deviations for at least three independent evaluations. Statistical analysis was performed using the SPSS software (SPSS Inc., Chicago, IL, USA). The differences between the mean values of multiple groups were analyzed using one-way analysis of variance (ANOVA) with Duncan's multiple range tests.

RESULTS

Effects of the extract on three immune cells

The practical chemical analysis revealed that the prepared extract had saccharide content of 81.82% on dry basis. The extract was used to assess its potentials to activate the three immune cells, and the obtained results are listed in table 1. In total, the extract behaved a dose-dependent manner to enhance lymphocyte proliferation, macrophage phagocytosis and NK cell activity significantly ($P<0.05$). At lower dose level of 10 µg/ml, the extract increased lymphocyte proliferation and NK cell activity about 50% and 11%, respectively. At higher dose level of 50 µg/ml, the extract enhanced lymphocyte proliferation and NK cell activity near 150% and 47%, respectively. The extract at dose levels of 10-50 µg/ml also resulted in 8-18% increases in the measured macrophage phagocytosis. These results indicated that the extract had *in vitro* immune activities towards the three immune cells (i.e., immune cells activation).

Effects of the extract on IL-2, IL-4 and IFN-γ secretion in splenocytes

Based on the data demonstrated in table 2, the extract had helpful stimulation on the excretion of IL-2 and IFN-γ in splenocytes, but showed inhibition on the excretion of IL-4. Totally, stimulation and inhibition of the extract on the three immune-related molecules behaved in a dose-dependent manner. In comparison with the assayed results from the control group using the sterilized water, the extract at dose level of 10 µg/ml could enhance IL-2 and IFN-γ secretion levels by 2% and 116%, respectively, and reduce IL-4 secretion level by 9%. At the same time, the extract at dose level of 50 µg/ml increased IL-2 and IFN-γ secretion levels by 18% and 272%, respectively and decreased IL-4 secretion level by 28%. These data demonstrated *in vitro* immunomodulatory properties of the extract again; that is, the extract was capable of stimulating IL-2 and IFN-γ secretion but inhibiting IL-4 secretion in splenocytes.

Effects of the extract on IL-1β, IL-6, TNF-α, iNOS and LZM secretion in macrophages

Based on these data listed in table 3, the extract had beneficial effects on the secretion of IL-1β, IL-6, TNF-α, iNOS and LZM in macrophages. Mostly, the extract showed promotion on IL-1β, IL-6 and TNF-α as well as stimulation on iNOS and LZM in a dose-dependent manner. In comparison with the control group, the extract at dose level of 10 µg/ml increased secretion levels of IL-1β, IL-6, TNF-α, iNOS and LZM about 32%, 13%, 50%, 29% and 23%, respectively. However, the extract at higher dose level of 50 µg/ml could enhance IL-1β, IL-6, TNF-α, LZM and iNOS secretion into much higher levels, with increases about 78%, 125%, 265%, 666% and 85%, respectively. These results once more declared that the extract had *in vitro* activities towards macrophages, bringing about significant secretion enhancements of five immune-related molecules (IL-1β, IL-6, TNF-α, iNOS and LZM).

DISCUSSION

Immune system is the ultimate defense of the organisms against many diseases. Immune function of natural polysaccharides may begin with activating effect on cells such as lymphocytes, macrophages and others. To clarify immune potentials of the soluble polysaccharides from *D. opposita*, a water-soluble polysaccharide extract was prepared and briefly purified by a two-step treatment in this study; after then, its *in vitro* effects on cell-mediated

Table 1: *In vitro* effects of the soluble polysaccharide extract on lymphocyte proliferation, macrophage phagocytosis and NK cell activity.

Dose levels of the extract (μg/ml)	Proliferation (A_{450})	Phagocytosis (A_{540})	Cell activity (%)
0	0.449±0.016[a]	0.146±0.033[a]	42.5±2.9[a]
10	0.673±0.027[b]	0.158±0.037[b]	47.0±2.9[b]
30	0.794±0.011[c]	0.166 ±0.025[bc]	57.5±2.3[c]
50	1.125±0.017[d]	0.173±0.042[c]	62.5±1.1[d]

Table 2: *In vitro* effects of the soluble polysaccharide extract on secretion levels of IL-2, IL-4 and IFN-γ.

Immune molecules	Dose levels of the extract (μg/ml)			
	0	10	30	50
IL-2 (pg/ml)	41.4±3.5[a]	42.2±3.6[a]	43.0±2.86[a]	48.8±3.4[b]
IL-4 (pg/ml)	38.9±4.5[b]	35.4±2.5[ab]	33.4±2.7[ab]	27.9±3.1[a]
IFN-γ (pg/ml)	24.6±3.4[a]	53.1±5.3[b]	59.6±3.51[bc]	91.5±6.2[c]

Table 3: *In vitro* effects of the soluble polysaccharide extract on secretion levels of IL-1β, IL-6, TNF-α, iNOS and LZM.

Immune molecules	Dose levels of the extract (μg/ml)			
	0	10	30	50
IL-1β (pg/ml)	73.4±2.3[a]	96.9±4.6[b]	103.8±4.1[b]	131.0±6.6[c]
IL-6 (pg/ml)	26.6±0.7[a]	30.1±3.6[ab]	40.2±6.1[b]	60.2±4.5[c]
TNF-α (pg/ml)	39.6±4.7[a]	59.4±2.0[b]	104.6±3.6[c]	144.7±5.8[d]
iNOS (pg/ml)	86.0±3.4[a]	197.2±7.4[a]	478.8±12.9[b]	658.5±14.9[b]
LZM (ng/ml)	17.8±2.9[a]	21.9±1.1[a]	28.6±1.1[b]	33.0±3.8[b]

Different lowercase letters after the mean values as the superscripts in same row indicate that one-way ANOVA of the mean values is different significantly (P<0.05).

immunity and mononuclear phagocytic system function were evaluated. The results showed that the extract was able to enhance macrophage phagocytosis, splenocyte proliferation and NK cell activity, and also able to regulate the secretion of cytokines and immune-related enzymes in two immune cells. These results proved immune function of the soluble polysaccharides of *D. opposita*. However, *in vivo* immune potentials of the soluble polysaccharides are still not assessed in the present time. An *in vivo* study is necessary in future study.

Natural polysaccharides have been widely investigated for their *in vitro* immune activities towards immune cells. *Pyracantha fortuneana* (Maxim.) Li polysaccharides show immune function by increasing NK cell activity from 55.7% to 72.9% (Yuan *et al*., 2010). *Astragalus* polysaccharide and *Astragalus membranaceus* polysaccharide both can enhance phagocytic capacity of macrophages (Zhang *et al*., 2010; Yang *et al*., 2013). The polysaccharides from *Sargassum fusiforme*, *astragalus* and Chinese *angelica* are also able to promote the proliferation of splenic lymphocytes by 86–200% (Zhang *et al*., 2010; Chen *et al*., 2012; Qin *et al.,* 2013). Moreover, the mucopolysaccharide separated from *D. opposita* shows immune function via enhancing NK cell activity in lymphocytes and activating macrophages (Choi *et al*., 2004). These mentioned results draw conclusion consistent with that of the present study; that is, the soluble polysaccharides from *D. opposite* had *in vitro* immune potentials.

Natural polysaccharides also have effects on the secretion of some immune-related molecules, including these immune-related enzymes and cytokines. Natural polysaccharides can activate iNOS to synthesize more NO (Lee *et al.,* 2006; Son *et al.,* 2006), or can enhance LZM activity in macrophages (Wu *et al.,* 2014).) The mucopolysaccharide from *D. opposita* was capable of promoting the secretion of nitric oxide (NO), IL-1, IFN-γ, and other immune-related molecules (Choi *et al*., 2004). The *Cheonggukjang* polysaccharides could increase IL-2 and IFN-γ production in splenocytes, and stimulate NO production in the studied Raw 264.7 macrophages (Lee *et al*., 2013). *Astragalus membranaceus* polysaccharide is able to increase the respective secretion of IL-2, IL-12 and TNF-α by 71%, 54% and 89%, but also able to decrease IL-10 secretion by 52% in serum (Yang *et al*., 2013). The water-soluble polysaccharide from *Sargassum fusiforme* can promote cytokines (IL-2, IL-6 and TNF-α) secretion of peritoneal macrophages by 50-250% (Chen *et al*., 2012). The mentioned results are consistent with these results of this study, supporting that the polysaccharide extract could increase the secretion of the seven immune-related molecules (IL-2, IFN-γ, TNF-α, IL-1β, IL-6, iNOS and LZM) but decrease the secretion of another immune-related molecule (IL-4).

Cytokines and immune cells both play prominent roles in the development of immune responses. Lymphocytes directly involve in cell-mediated immunity (Im *et al*., 2006), while one form of the cell-mediated immunity is to secrete cytokines. Activated splenic lymphocytes thereof play important role in the body, by producing cytokines such as IL-2, IL-4, IL-6, IL-10 and IFN-γ (Chen *et al*., 2012). Macrophages carry out phagocytosis, stimulate lymphocytes or other immune cells to produce cytokines such as IL-1, IL-3, IL-6, IFN-α and TNF-α and promote the formation of NO and iNOS after being activated (Yoon *et al*., 2004). The possible mechanism related to immune function of natural polysaccharides is thus suggested as activating immune cells and secreting more immune-related molecules (Choi *et al*., 2004; Kim *et al*., 2011). This is to say, natural polysaccharides will activate some immune cells firstly, which then secrete more immune molecules. The respective results in this study are also consistent with this suggested mechanism.

CONCLUSION

The water-soluble polysaccharide extract from *D. opposita* had immune potentials to the body. In a dose-dependent manner, it could activate three immune cells (macrophages, NK cells and splenocytes), enhance the secretion of seven immune-related molecules (IL-2, IFN-γ, TNF-α, IL-1β, IL-6, iNOS and LZM), but inhibit the secretion of another immune molecule (IL-4). These obtained results indicated that the possible mechanism related to the immune function of the soluble *D. opposita* polysaccharides was to activate immune cells and to regulate the secretion of immune-related molecules.

ACKNOWLEDGMENTS

This study was funded by the Specialized Research Fund for the Doctoral Program of Higher Education (Project No. 20092325110012). The authors also thank the anonymous reviewers and editors for their valuable advices.

REFERENCES

Calder PC, Susanne KE and Esther C (2006). Early nutrition and immunity-progress and perspectives. *Brit. J. Nutr*., **96**: 774-790.

Chen XM, Nie WJ, Fan SR, JF, Zhang, Wang YX, Lu JX and Jin LQ (2012). A polysaccharide from *Sargassum fusiforme* protects against immunosuppression in cyclophosphamide-treated mice. *Carbohyd. Polym*., **90**: 1114-1119.

Choi EM, Koo SJ and Hwang JK (2004). Immune cell stimulating activity of mucopolysaccharide isolated from yam (*Dioscorea batatas*). *J. Ethnopharmacol*., **91**: 1-6.

Dubosi M, Gilles KA and Hamilton JK (1951). A colorimetric method for the determination of sugars. *Nature*, **28**: 167-168.

Dunn GP, Old LJ and Schreiber RD (2004). The immunobiology of cancer immunosurveillance and immunoediting. *Immunity*, **21**: 137-148.

Farombi EO, Britton G and Emerole GO (2000). Evaluation of the antioxidant activity and partial characterization of extracts from browned yam flour diet. *Food Res. Int*., **33**: 493-499.

Hriram V, Jahagirdar S, Latha C, Kumar V, Puranik V and Rojatkar S (2008). A potential plasmid curing agent, 8-epidiosbulbin E acetate, from *Dioscorea bulbifera* L. against multi drug resistant bacteria. *Int. J. Antimicrob. Agr*., **32**: 405-410.

Hsu JH, Wu YC, Liu IM and Cheng JT (2007). Dioscorea as the principal herb of Die-Huang-Wan, a widely used herbal mixture in China, for improvement of insulin resistance in fructose-rich chow-fed rats. *J. Ethnopharmacol*., **25**: 577-584.

Hsu SY, Hua KF, Lin CC, Lin CH, Hsu J and Wong CH (2004). Extract of Reishi polysaccharides induces cytokine expression via TRL4-modulated protein kinase signaling pathways. *J. Immunol*., **173**: 5989-5999.

Hussein MMD, Helmy WA and Salem HM (1998). Biological activities of some galactomannans and their sulfated derivatives. *Phytochem*., **48**: 479-484.

Im SA, Kim K and Lee CK (2006). Immunomodulatory activity of polysaccharides isolated from *Salicornia herbacea*. *Int. Immunopharmacol*., **6**: 1451-1458.

Kim JK, Cho ML, Karnjanapratum S, Shin IS and You SG (2011). *In vitro* and *in vivo* immunomodulatory activity of sulfated polysaccharides from *Enteromorpha prolifera*. *Int. J. Biol. Macromol*., **49**: 1051-1058.

Lee KY, Lee MH, Chang IY, Yoon SP, Lima DY and Jeon YJ (2006). Macrophage activation by polysaccharide fraction isolated from *Salicornia herbacea*. *J. Ethnopharmacol*., **103**: 372-378.

Lee SJ, Rim HK, Jung JY, An HJ, Shin JS, Cho CW, Rhee YK, Hong HD and Lee KT (2013). Immunostimulatory activity of polysaccharides from *Cheonggukjang*. *Food Chem. Toxicol*., **59**: 476-484.

Leung MYK, Liu C, Koon JCM and Fung KP (2006). Polysaccharide biological response modifiers. *Immunol. Lett*., **105**: 101-114.

Liu F, Ooi VEC and Chang ST (1997). Free radical scavenging activities of mushroom polysaccharide extracts. *Life Sci*., **60**: 763-771.

Liu SY, Wang JY, Shyu YT and Song LM (1995). Studies on yam (*Dioscorea spp*.) in Taiwan. *J. Chin. Med*., **6**: 111-126.

Ooi VE and Liu F (2000). Immunomodulation and anti-cancer activity of polysaccharide-protein complexes. *Curr. Med. Chem*., **7**: 715-729.

Peters D (2006). Carbohydrates for fermentation. *Biotech. J*., **1**: 806-814.

Qin T, Chen J, Wang DY, Hu YL, Zhang J, Wang M, Qiu SL, Gao ZZ, Liu RR, Yu Y, Huang Y, Wang QC and Wang QX (2013). Selenylation modification can enhance immune-enhancing activity of Chinese angelica polysaccharide. *Carbohyd. Polym*., **95**: 183-187.

Roman BJA and Jimenez SA (2006). NF-kappa B as a potential therapeutic target in osteoarthritis and rheumatoid arthritis. *Osteoarthr. Cartilage*, **14**: 839-848.

Saima Y., Das AK, Sarkar KK, Sen AK and Sur P (2000). An antitumor pectic polysaccharide from *Feronia limoni*a. *Int. J. Biol. Macromol*., **27**: 333-335.

Scheppach W, Luehrs H, Melcher R, Gostner A, Schauber J and Kudlich T (2004). Antiinflammatory and anticarcinogenic effects of dietary fibre. *Clin. Nutr. Suppl*., **1**: 51-58.

Sideras K, Braat H and Kwekkeboom J (2014). Role of the immune system in pancreatic cancer progression and immune modulating treatment strategies. *Cancer Treat. Rev*., **4**: 513-522.

Son CG, Shin JW, Cho HJ, Cho CK, Yun CH, Chung W and Han SH (2006). Macrophage activation and nitric oxide production by water soluble components of *Hericium erinaceum*. *Int. Immunopharmacol*., **6**: 1363-1369.

Son IS, Kim JH, Sohn HY, Son KH, Kim JS and Kwon CS (2007). Antioxidative and hypolipidemic effects of diosgenin, a steroidal saponin of yam (*Dioscorea spp*.) on high-cholesterol fed rats. *Biosci*. *Biotech*. *Bioch*., **71**: 3063-3071.

Tzianabos A, Wang JY and Kasper DL (2003). Biological chemistry of immunomodulation by zwitterionic polysaccharides. *Carbohyd. Res*., **338**: 2531-2538.

Wu GH, Lu CL, Huang ZL, Li ZY and Jiang JG (2014). Regulation effect of polysaccharides from *Pleurotus tuber-regium* (Fr.) on the immune activity of mice macrophages. *Food Funct*., **5**: 337-344.

Yamada H (1994). Pectic polysaccharides from Chinese herbs: Structure and biological activity. *Carbohyd. Polym*., **25**: 269-276.

Yang B, Xiao B and Sun TY (2013). Antitumor and immunomodulatory activity of *Astragalus membranaceus* polysaccharides in H22 tumor-bearing mice. *Int. J. Biol. Macromol*., **62**: 287-290.

Yoon YD, Kang JS, Han SB, Park SK, Lee HS, Kang JS and Kim HM (2004). Activation of mitogen-activated protein kinases and AP-1 by polysaccharide isolated from the radix of *Platycodon grandiflorum* in RAW 264.7 cells. *Int. Immunopharmacol*., **4**: 1477-1487.

Yuan CF, Wang CD, Bu YQ, Xiang TX, Huang XN, Wang ZW, Yi FP, Ren GS, Liu GL and Song FZ (2010). Antioxidative and immunoprotective effects of *Pyracantha fortuneana* (Maxim.) Li polysaccharides in mice. *Immunol. Lett*., **133**: 14-18.

Zava DT, Dollbaum CM and Blen M (1998). Estrogen and progestin bioactivity of foods, herbs, and spices. *P. Soc. Exp. Bio. Med*., **217**: 369-378.

Zhang NW, Li JF, Hu YX, Cheng GL, Zhu XY, Liu FQ, Zhang YJ, Liu ZJ and Xu JQ (2010). Effects of astragalus polysaccharide on the immune response to foot-and-mouth disease vaccine in mice. *Carbohy. Polym*., **82**: 680-686.

Zhu XL and Lin ZB (2005). Effects of *Ganoderma lucidum* polysaccharides on proliferation and cytotoxicity of cytokine-induced killer cells. *Acta. Pharmacol. Sin*., **26**: 1130-1137.