

Modulation of cytokine production and complement activity by biopolymers extracted from medicinal plants

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Abstract

This study evaluates the anti-inflammatory activities of the extracts obtained from the medicinal plants *Aster tataricus*, *Pinellia ternata*, *Ostericum koreanum*, *Platycodon grandiflorum*, *Asarum sieboldii*, *Tussilage farfara*, and *Acanthopanax sessiliflorus*. These plants are used in Korea to treat inflammatory and respiratory ailments, including asthma. The hot water extract (HWE) of *A. sessiliflorus* inhibited production of IFN- γ , IL-2, IL-4 and IL-5. The HWE of *A. tataricus*, *O. koreanum*, *P. grandiflorum*, *A. sieboldii* and *T. farfara* had similar inhibitory effects on IFN- γ and IL-5. The endo-polysaccharide (ENP) screened were less active than the HWE and had varying effects on the production of cytokines. The HWE fractions from all the plants except that from *P. grandiflorum* had marked anti-complement activity at a concentration of 1000 $\mu\text{g/ml}$. The ENP of *A. sieboldii*, *T. farfara* and *A. sessiliflorus* exhibited significant anti-complement activity compared to the positive control.

Keywords: Endo-polysaccharide (ENP); Anti-inflammatory; Cytokines; Complement

Introduction

The worldwide increase in inflammatory and allergic diseases has lead to an extensive search for new anti-inflammatory and immunosuppressive agents (Patwardhan, et al., 1990). In recent years, attention has focussed on polysaccharides isolated from fungi, algae, and plants because of their immunomodulatory activities (Ooi and Liu, 2000).

Inflammatory and allergic responses are regulated by the balance of pro- and anti-inflammatory cytokines in tissues (Barnes, 2002; Calixto, et al., 2004). T helper lymphocytes

(Th1 and Th2 cells) coordinate the cell-mediated immune/inflammatory responses that follow tissue insult or allergen exposure by releasing cytokines. The cytokine interferon γ (IFN- γ) is one of the major cytokines produced by Th1 cells in response to viral infections and it is a potent activator of other T and B lymphocytes, phagocytic cells and natural killer cells involved in eliminating them. Interleukin-4 (IL-4) and interleukin-5 (IL-5) are secreted by Th2 cells and mediate many of the responses observed in response to Helminth infections and inflammatory and allergic diseases (Kang, et al., 2004). IL-4 is central to B cell switching to IgE antibody production (Arthur and Mason, 1986) which underlies allergic inflammatory diseases (Tepper, et al., 1990; Ricci, et al., 1997). The cytokine IL-5 induces eosinophilia (Sanderson, 1992) and so has been a target in asthma (Leckie, et al., 2000).

The complement system plays a significant role in host defence. The complement cascade can be activated by antibody-antigen complexes (classical pathway), direct binding to the surface of some infectious agents (alternative pathway), or the MBL/MASP (mannan binding lectin/MBL-associated serine protease) pathway (Kirschfink, 1997). The 30 or more complement fragments that make up the complement system include proteolytic proenzymes, non-enzymatic components, co-factors, regulators and receptors (Ember and Hugli, 1997). Therefore modulation of complement activity may be useful in the therapy of inflammatory diseases. A number of compounds from plant and/or microbial origin have been reported to modulate the complement cascade (Locher, et al., 1995, Hildebert and Jordan, 1998, Park, et al., 2004, Lee, et al., 2005).

As part of a search for novel compounds derived from plants for use as modulators of inflammation, we have tested the effects on cytokine production (IFN- γ , IL-2, IL-4 and IL-5) and complement activity of the extracts from the medicinal plants *Aster tataricus* (S1), *Pinellia ternata* (S2), *Ostericum Koreanum* (S3), *Platycodon grandiflorum* (S4), *Asarum sieboldii* (S5), *Tussilage farfara* (S6), and *Acanthopanax sessiliflorus* (S7). These are widely used in traditional medicine in China and Korea for treating inflammation in airways. *Aster tataricus* is used in traditional Chinese medicine for the relief of coughs and it possesses diuretic, antitumor, antibacterial, antiviral and anti-ulcer activities (Morita et al., 1996; Shao et al., 1997; Shirota et al., 1997). *Pinellia ternate*, *Platycodon grandiflorum* and *Tussilage farfara* possess anti-tumor and anti-bacterial properties (Cho, 2010., Chen et al., 2003., Franz, 1969, Kim et al., 2006). *Asarum sieboldii* is reported to contain anti-allergic compounds (Hashimoto et al., 1994). The shoot and roots of various species of *Acanthopanax* (Araliaceae) have long been used as traditional medicine for many ailments, including diabetes, neuralgia, palsy, gastric ulcer, learning-behavior difficulties, and cancer in China, Korea, and Japan (Han et al., 1998; Yook et al., 1996; Fujikawa et al., 1996, Jeong et al., 2007). These plants are used when exhaling air from the lungs becomes difficult, which causes the wheezing and coughing that is associated with asthma. The most common method of taking these herbs is as a water decoction to strengthen the immune system and/or reduce the severity of an asthma attack (Wang, 2001; Ding, 2000). Based on traditional practice, in this study hot water extracts are used for testing the bioactivities of these plants. Generally the hot water extracts consist of polysaccharides, proteins and amino acids, minerals, sugars, phenolics, flavonoids and other water soluble organic compounds. The endo-polysaccharides are obtained by the precipitation of hot water extracts with ethanol and predominantly contain sugars, with small amounts of protein. The purpose of this study is to systematically evaluate the modulation of cytokine production and complement activity of the hot water extracts and isolate endo-

polysaccharides from the hot water extracts of seven traditional medicinal plants used for the treatment of inflammation in airways and asthma.

Materials and methods

Plant materials

Aster tataricus L. (Bacteriaceae), *Pinellia ternata* Breit. (Araceae), *Ostericum koreanum* Kitagawa. (Umbelliferae), *Platicodon grandiflorum* A. DC. (Campanulaceae), *Asarum sieboldii* Miquel var. (Aristolochiaceae), *Tussilage farfara* L. (Asteraceae), and *Acanthopanax sessiliflorus* Rupr. & Maxim. (Araliaceae) were purchased from local herb and drug markets in South Korea. These plants are grown in Korea, China and other parts of Asia.

Reagents and chemicals

Ficoll-plaque was purchased from Pharmacia Biotech AMRAD (Uppsala, Sweden) and foetal bovine serum (FBS) from Hyclone (Logan, UTAH, USA), Roswell Park Memorial Institute-1640 (RPMI-1640) medium containing 25 mM HEPES, phosphate buffered saline (PBS) and gentamycin were from Thermo Electron (Melbourne, Australia). Phytohaemagglutinin-L (PHA), HEPES, Hank's balanced salts solution (HBSS), Triton X-100, 20 were from Sigma (St Louis, Missouri, USA). Dulbecco's modified Eagle's medium (DMEM), penicillin-streptomycin and Amphotericin B were from GIBCO BRL (USA). Dialysis tubing (MW cut-off; 6,000-8,000 Da) was from Spectrum Laboratories, Inc. (USA). All other chemicals were of A.R. grade. ELISA kits for IL-2, IL-4, IL-5 and IFN- γ were from R&D systems, Minneapolis (USA). Tetramethylbenzidine (TMB) solution was from KPL (Gaithersburg, Maryland, USA). Goat anti-human C3 was purchased from Sigma Co., and IgM hemolysin sensitized sheep erythrocyte (EA) from Lyophilization Laboratory Inc. (Japan). 5.5'-Barbituric acid sodium salt was purchased from Merk Co. The other chemicals were reagent grade and obtained from commercial sources. Normal human serum (NHS) was obtained from healthy adults with their informed consent.

Preparation of hot water extracts (HWE) and endo-polysaccharide (ENP)

The preparation and isolation of HWEs and ENPs from the plants was as described previously (Jeong, et al., 2006) and is summarised in Figure 1. The dried herb (50 g) was cut into the small pieces and autoclaved for 2 h with 10 times its volume of water. The yield of each HWEs and ENPs is shown in Table 1.

Peripheral blood mononuclear cell isolation, culture and treatment

To assess the effects of the extracts on cytokine production, human peripheral blood mononuclear cells (PBMCs) from healthy adult donors were purified from anticoagulated blood by discontinuous density gradient centrifugation with Ficoll-Paque. The PBMCs were plated onto 24 well plates at 1×10^6 cells/ml in RPMI-1640 supplemented with 5% heat-inactivated FBS, 25 mM HEPES, 2mM L-glutamine and gentamycin at 20 μ g/ml. PBMC cultures were left untreated or treated in triplicate with extracts from the plants at 500 μ g/ml. They were also activated with PHA at 1 μ g/ml, or left unstimulated and cultured in a humidified 5%

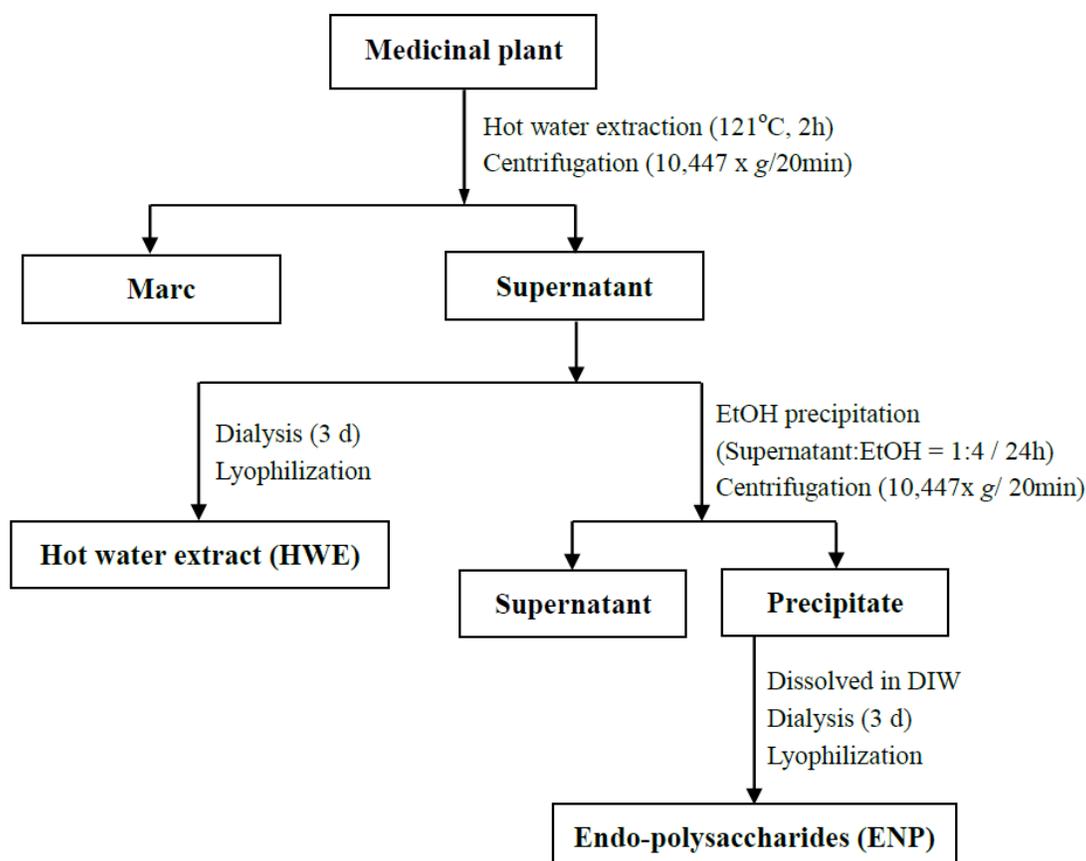


Figure 1. Schematic diagram for the processing of hot water extracts (HWEs) and endo-polysaccharides (ENPs) from medicinal plants.

CO₂ in air atmosphere at 37⁰ C. After 44 h incubation the cultures were harvested and stored at -80⁰C prior to the determination of their cytokine content.

Measurement of PBMC cytokine production

PBMC production of IFN- γ , IL-2, IL-4 and IL-5 in the cell cultures was assessed by ELISA using commercial kits specific for each of the cytokines and following the protocols provided by the manufacturer.

Assay for complement activity

The effect of the extracts on complement activity was measured by the complement fixation test which is based on complement consumption by test samples and degree of red blood cell lysis by the residual complement (Kabat and Mayer, 1964). Fifty μ l of extract solution in water was mixed with equal volumes of normal human serum (NHS) and GVB (gelatin veronal buffered saline, pH 7.4) containing 500 μ g Mg⁺⁺ and 150 μ g Ca⁺⁺. The mixtures were incubated at 37⁰C for 30 min and the residual total complement hemolysis (TCH₅₀) was determined by adding IgM hemolysin sensitized sheep erythrocytes at 1×10^8 cells/ml. The NHS was incubated with deionized water (DIW) and GVB⁺⁺ to provide a control. The LPS

was used as positive control. The effect of the extracts on complement activity was expressed as the percentage inhibition of the TCH₅₀ of control (Jeong, et al., 2004).

Data analysis

Average results for cytokine release or anti-complement activity were determined from triplicate cultures/wells. Mean cytokine release data was determined from the averaged results for each treatment from all the blood donors. Any treatment-induced differences were analysed to determine if they were statistically significant using Duncan's multiple-range test. Significance was defined as $p < 0.05$.

Results

The yields of hot water extracts and endo-polysaccharides varied widely for the samples and the results are presented in Table 1. The yield of the water extracts was in the range of 91.85–225.67 mg/10g whereas the endo-polysaccharides lie in the range 17.31 to 78.98 mg/10g.

Effects of HWE and ENP of medicinal plants on production of inflammatory cytokines by human PBMC

The effects of HWE on cytokine production by activated human T lymphocytes are shown in Figure 2. We have examined the *in vitro* effects of HWE on the secretion of Th1 and

Table 1. List of medicinal herbs used in this study and their yields in hot water extracts and endo-polysaccharides.

Plant name (Family)	Medicinal uses	Plant part used	mg / 10g of dried plant	
			Hot water extracts (HWEs)	Endo-polysaccharides (ENPs)
<i>Aster tataricus</i> L. (Bacteriaceae)	Antitumor, antibacterial, antiviral and anti-ulcer activities (Morita et al., 1996)	Root	91.85	18.18
<i>Pinellia ternata</i> Breit. (Araceae)	Anti-tumor (Cho, 2010), antimicrobial and anti-inflammatory (Chen., et al., 2003)	Stem	135.21	33.80
<i>Ostericum koreanum</i> Kitagawa (Umbelliferae)	Anti-allergic (Lung et al., 2011), Anti-tumor (Kang et al., 2009)	Root	82.23	27.95
<i>Platycodon grandiflorum</i> A. DC. (Campanulaceae)	Anti-cancer (Huang et al., 2008)	Root	178.61	33.93
<i>Asarum sieboldii</i> Miquel var. (Aristolochiaceae)	Anti-allergic (Hashimoto., et al., 1994)	Root	64.13	17.31
<i>Tussilage farfara</i> L. (Asteraceae)	Anti-tumor (Franz, 1969), anti-microbial and antioxidant (Kim et al., 2006)	Flower	225.67	78.98
<i>Acanthopanax sessiliflorus</i> Rupr. & Maxim. (Araliaceae)	Diabetes, neuralgia, palsy, gastric ulcer, learning-behavior difficulties, and cancer (Fujikawa et al., 1996), anti-complement activity (Jeong et al., 2007).	Bark	155.39	23.13

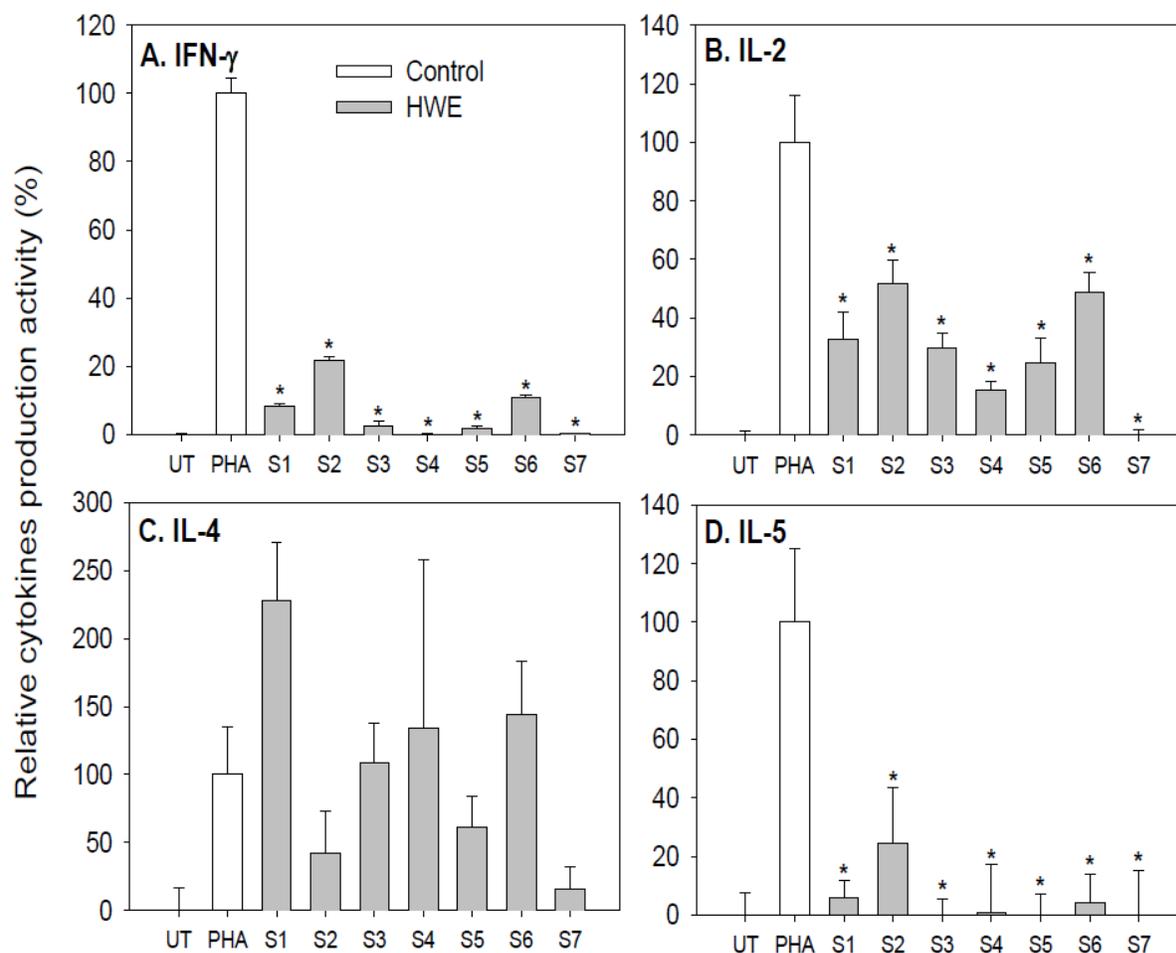


Figure 2. The effects of hot water extracts (HWEs) extracted from medicinal plants on cytokine secretion by PHA-activated human PBMC. Data shown are mean \pm S.D., expressed as a percentage of PHA control. UT: untreated; PHA: Phytohemagglutinin; S1: *Aster tataricus*; S2: *Pinellia ternata*; S3: *Ostericum Koreanum*; S4: *Platycodon grandiflorum*; S5: *Asarum sieboldii*; S6: *Tussilage farfara*; S7: *Acanthopanax sessiliflorus*. *Significant difference from control (PHA treated group), n=3, P<0.05.

and Th2 cytokines by human PBMC following activation with the T-lymphocyte mitogen PHA (Barrett, 1988). Detectable IL-2, IFN- γ and IL-5 were produced by the PHA-stimulated PBMC from all 3 donors. IL-4 production was detectable in PBMC from 2 of the 3 donors (Figure 2).

Many of the HWEs affected the production of Th1 (IFN- γ and IL-2) and Th2 (IL-4 and IL-5) cytokines by PHA-stimulated PBMC. The HWEs from *A. tataricus* (S1), *O. koreanum* (S3), *P. grandiflorum* (S4), *A. sieboldii* (S5), *T. farfara* (S6), and *A. sessiliflorus* (S7) inhibited the production of IFN- γ and IL-5 by >90% and *P. ternata* (S2) by ~80% (Figure 2A & D). The HWEs of *P. grandiflorum* (S4) and *A. sessiliflorus* (S7) also inhibited IL-2 production to a similar extent (>90% inhibition) but the HWEs from the other plants were less effective, inhibiting production by 50-70% (Figure 2B). Clear trends were observed for inhibition of IL-4 production with the HWEs of *P. ternata* (S2) and *A. sessiliflorus* (S7) and those from *A. tataricus* (S1) increased IL-4 production compared to the PHA control (Figure 2C).

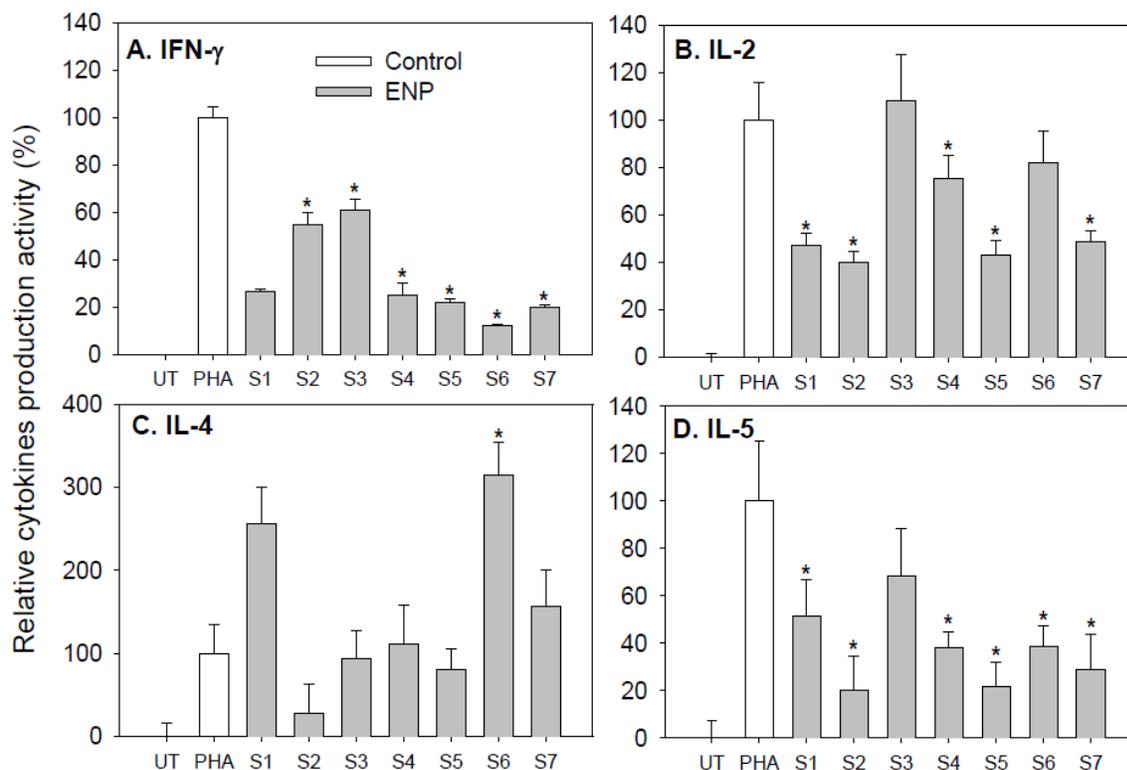


Figure 3. The effects of endo-polysaccharides (ENPs) extracted from medicinal plants on cytokine secretion by PHA-activated human PBMC. Data shown are mean \pm S.D., expressed as a percentage of PHA control. UT: untreated; PHA: Phytohemagglutinin; S1: *Aster tataricus*; S2: *Pinellia ternata*; S3: *Ostericum Koreamum*; S4: *Platycodon grandiflorum*; S5: *Asarum sieboldii*; S6: *Tussilage farfara*; S7: *Acanthopanax sessiliflorus* *Significant difference from control (PHA treated group), n=3, P<0.05.

The effects of ENPs extracted from the same medicinal plants on cytokine production by activated T lymphocytes are presented in Figure 3. Production of the Th1 cytokine IFN- γ was decreased by all ENP and the inhibition was >70% in the presence of *A. tataricus* (S1), *P. grandiflorum* (S4), *A. sieboldii* (S5), *T. farfara* (S6), and *A. sessiliflorus* (S7) ENPs. The production of IL-2 was not significantly affected by *O. koreamum* (S3) and *T. farfara* (S6) but it was reduced by >50% by the S1, S2, S5 and S7 ENPs.

Treatment with ENPs from *P. ternata* (S2), *P. grandiflorum* (S4), *A. sieboldii* (S5), *T. farfara* (S6) and *A. sessiliflorus* (S7) reduced production of the Th2 cytokine IL-5 by the PHA-stimulated PBMC by 50% or more. Only the *O. koreamum* (S3) ENPs had no significant effect. In contrast, clear trends towards increased levels of IL-4 were observed in *A. tataricus* (S1) and *T. farfara* (S6) treated samples and only in *P. ternata* (S2) treated samples were IL-4 levels reduced.

Effects of HWEs and ENPs of medicinal plants on complement activity

Anti-complementary activity is an immunomodulating activity that is responsible for immunological defence. The complement system plays an important role in host defence against foreign invasive organisms such as bacteria, fungi and viruses (Yamagishi, et al., 2003).

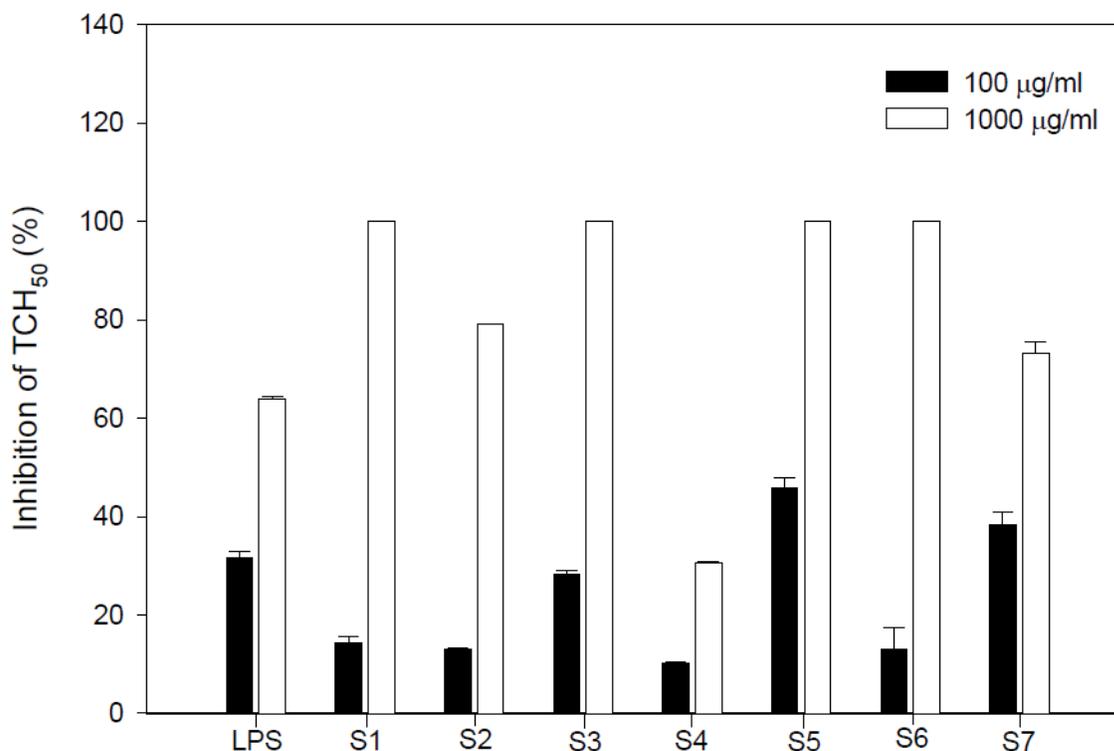


Figure 4. Anti-complementary activities of hot water extracts (HWEs) obtained from seven kinds of medicinal plants. LPS: positive control (lipopolysaccharide from *Escherichia coli* 0127: B8). S1: *Aster tataricus*; S2: *Pinellia ternata*; S3: *Ostericum Koreamum*; S4: *Platycodon grandiflorum*; S5: *Asarum sieboldii*; S6: *Tussilage farfara*; S7: *Acanthopanax sessiliflorus*

The extract of plants was investigated for complement activation through the inhibition of total complement haemolysis (ITCH₅₀ %). We also studied the effects of HWEs and ENPs isolated from these seven medicinal plants on complement activity. The effects of the extracts were studied at two concentrations (100 and 1000 µg/ml) and the results are presented in Figures 4 and 5. The effects of the HWEs on complement activity were concentration-dependent. For all the HWEs except that from *P. grandiflorum* (S4), the ITCH₅₀ value at this concentration was higher than the positive (LPS) control sample. The HWEs at the lower concentration (100µg/ml) mostly promoted complement activity as well. Only three either had no effect (*O. koreamum*: S3), or were slightly lower (*A. sieboldii*: S5; *A. sessiliflorus*: S7) than the LPS control at the same concentration.

Complement activity was increased by treatment with *A. sieboldii* (S5), *T. farfara* (S6) and *A. sessiliflorus* (S7) ENPs at the higher concentration (1000µg/ml). Other treatments had lower activities, especially in the case of *A. tartaricus* (S1), *O. koreamum* (S3) and *P. grandiflorum* (S4) as shown in Figure 5.

Discussion

Medicinal plants play a major role in the lives of many people throughout the world, and their use in western countries has increased significantly in recent years. *Aster tataricus* (S1), *Pinellia ternata* (S2), *Ostericum koreanum* (S3), *Platycodon grandiflorum* (S4), *Asarum*

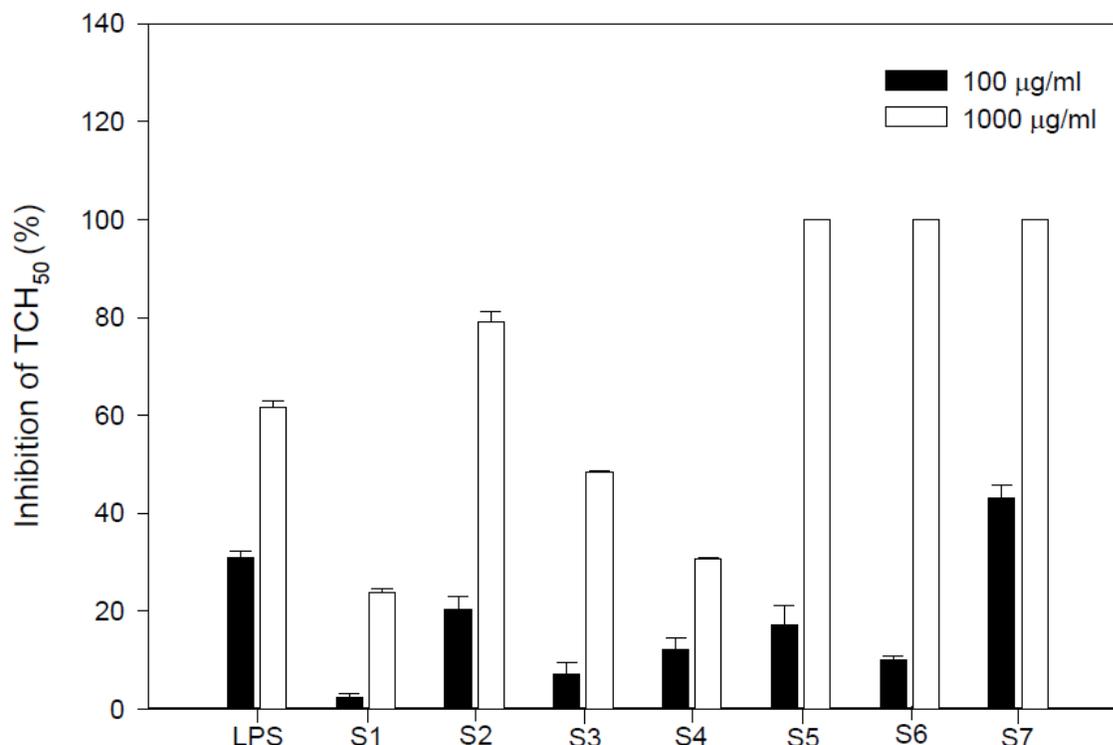


Figure 5. Anti-complementary activities of endo-polysaccharides (ENPs) extracted from seven kinds of medicinal plants. LPS: positive control (lipopolysaccharide from *Escherichia coli* 0127: B8). S1: *Aster tataricus*; S2: *Pinellia ternata*; S3: *Ostericum Koreamum*; S4: *Platycodon grandiflorum*; S5: *Asarum sieboldii*; S6: *Tussilage farfara*; S7: *Acanthopanax sessiliflorus*

sieboldii (S5), *Tussilage farfara* (S6), and *Acanthopanax sessiliflorus* (S7) are widely used traditional medicines for treating asthma. This study provides evidence that HWEs and ENPs extracted from plants S1-S7 have immunomodulatory activities *in vitro*. The HWEs from the plants inhibited cytokine production more than their corresponding ENPs. This is especially so for the Th1 cytokine IFN- γ and the Th2 cytokine IL-5. The HWEs consist of ENPs and other highly polar water soluble compounds. The HWEs of *A. sessiliflorus* (S7) virtually eliminated all PBMC cytokine production.

The HWEs at the same concentration generally increased complement activity also. Only the HWEs and ENPs of *P. grandiflorum* (S4) showed lower complement activity at all concentrations tested.

The effects of these plant extracts on the production of Th1 and Th2 cytokines by PBMC were studied because of the putative roles of these cytokines in health and disease. The Th1 cytokine IFN- γ negatively regulates IL-4 and IL-5 production (Romagnani, et al., 1989; Maggi, et al., 1992; Finkelman, et al., 1988). Thus they can prevent the IL-4 dependent switch of B-lymphocytes to IgE production that underlies allergic reactions, as well as IL-5-induced maturation, recruitment and activation of eosinophils (Nakajima, et al., 1993). Eosinophilic inflammation is a characteristic feature of asthma and is thought to result from an imbalance in the level of these Th1 and Th2 cytokines.

In this study, we attempted to evaluate the effects of HWEs and ENPs of medicinal plants on the total complement system. The HWEs of all samples except *P. grandiflorum* (S4) clearly exhibited anti-complement activity when used at a high concentration. The ENPs of *A. sieboldii* (S5), *T. farfara* (S6) and *A. sessiliflorus* (S7) also contained significant anti-complement activity. This pattern of complement inhibition by extracts from plants was similar to that of various polymeric substances of *Angelica acutiloba* (Yamada, et al., 1984; Kiyohara, et al., 1989), *Artemisia princeps* (Yamada, et al., 1985), *Alcaligenes faecalis* (Honda, et al., 1986) and *Azadirachta indica* (Van der Nat, et al., 1989).

The findings of the present study identify possible ways in which the water extracts of many of these plants may have some beneficial anti-inflammatory effects for the treatment of asthma. However further *in vitro* and *in vivo* pharmacological studies are required to establish this. Our current research is focussed on the isolation and structure elucidation of the active compounds from the HWEs, together with a detailed analysis of the modulation of cytokines and complement activity.

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Conflict of Interest

There is no conflict of interest associated with the authors of this paper, and the funding sponsor did not exert any inappropriate influence on this work.

References

- Arthur RP, Mason D. (1986). T cells that help B cell responses to soluble antigen are distinguishable from those producing interleukin 2 on mitogenic or allogeneic stimulation. *The Journal of experimental medicine* 163, 774-786.
- Barnes PJ. (2002). Cytokine modulators as novel therapies for asthma. *Annual review of pharmacology and toxicology* 42, 81-98.
- Barrett JT. (1988). *Textbook of Immunology*, Mosby, St.Louis, MO, USA.
- Calixto JB, Campos MM, Otuki MF, Santos ARS. (2004). Anti-inflammatory compounds of plant origin. Part II. Modulation of pro-inflammatory cytokines, hemokines and adhesion molecules. *Planta Medica* 70, 93-103.
- Chen J, Cui G, Liu J, Tan R. (2003). Pinelloside, an antimicrobial cerebroside from *Pinellia ternata*. *Phytochemistry* 64, 903-906.
- Cho WCS. (2010). Supportive cancer care with Chinese medicine, Hongkong: Springer Verlag
- Ding, Q. (2000), Discussion on Several Types of Reform of Chinese Medicine Decoction. *Lishizhen Medicine and Materia Medica Research*, 11, 26.
- Ember JA, Hugli TE. (1997). Complement factors and their receptors. *Immunopharmacology* 38, 3-15.
- Fujikawa T, Yamaguchi A, Morita I, Takeda H, Nihshibe S. (1996). Protective effects of *Acanthopanax senticosus* Harms from Hokkai do and its components on gastric ulcer

- in restrained cold water stressed rats. *Biological and Pharmaceutical Bulletin* 19, 1227-1230.
- Finkelman FD, Katona IM, Mosmann TR, Coffman RL. (1988). IFN-gamma regulates the isotypes of Ig secreted during in vivo humoral immune response. *Journal of Immunology* 140, 1022-1027.
- Franz G. (1969). Studies on the mucopolysaccharides of *Tussilago farfara* L., *Symphytum officinalis* L., *Borago officinalis* L. and *Viola tricolor* L. *Planta Medica*. 17, 217-220.
- Han SB, Kim YH, Lee CW, Park SM, Lee HY, Ahn KS, Kim HM. (1998). Characteristic immunostimulation by angelan isolated from *Angelica fifas* Nakai. *Immunopharmacology* 40, 39-48.
- Hashimoto K, Yanagisawa T, Okui Y, Ikeya Y, Maruno M, Fujita T. (1994). Studies on anti-allergic components in the roots of *Asiasarum sieboldi*. *Planta Medica* 60, 124-127.
- Hildebert W, Jordan E. (1988). An immunologically active arabinogalactan from *Viscum album* berries. *Phytochemistry* 27, 2511-2517.
- Honda S, Sugino H, Asano T, Kakinuma A. (1986). Activation of the alternative pathway of complement by an antitumor (1,3)- β -D-glucan from *Alcaligenes faecalis* var. *myxogenes* IFO 13140, and its lower molecular weight and carboxymethylated derivatives. *Immunopharmacology* 11, 29-37.
- Jeong SC, Yang BK, Ra KS, Wilson MA, Cho Y, Gu YA, Song CH. (2004). Characteristics of anti-complementary biopolymer extracted from *Coriolus versicolor*. *Carbohydrate Polymer* 55, 255-263.
- Jeong SC, Jeong YT, Yang BK, Song CH. (2006). Chemical characteristics and immuno-stimulating properties of biopolymers extracted from *Acanthopanax sessiliflorus*. *Journal of Biochemistry and Molecular Biology* 39, 84-90.
- Jeong SC, Yang BK, Jeong YT, Rao KS, Song CH. (2007). Isolation and characterization of biopolymers extracted from the bark of *Acanthopanax sessiliflorus* and their anticomplement activity. *Journal of Microbiol Biotechnology* 17, 21- 28.
- Jung HW, Jung JK, Park YK. (2011). Antiallergic effect of *Ostericum koreanum* root extract on ovalbumin-induced allergic rhinitis mouse model and mast cells. *Asian Pacific Journal of Allergy Immunol and Immunology* 29, 338-348
- Kabat EE, Mayer MM. (1964). Complement and complement fixation. In C. C. Tomas (Ed.), *Experimental immunochemistry* Illinois, pp. 133-240.
- Kang TJ, Lee SY, Singh RP, Agarwal R, Yim DS. (2009). Anti-tumor activity of oxypeucedanin from *Ostericum koreanum* against human prostate carcinoma DU145 cells. *Acta Oncol*. 48, 895-900.
- Kang H, Ahn KS, Cho C, Bae HS. (2004). Immunomodulatory effect of *Astragali radix* extract on murine Th1/Th2 cell lineage development. *Biological and pharmaceutical bulletin* 27, 1946-1950.
- Kim MR, Lee JY, Lee HH, Aryal DK, Kim YG, Kim SK, Woo ER, Kang KW. (2006). Antioxidative effects of quercetin-glycosides isolated from the flower buds of *Tussilago farfara* L. *Food and Chemical Toxicology* 44, 1299-1307.
- Kirschfink M. (1997). Controlling the complement system in inflammation. *Immunopharmacology* 38, 51-62.
- Kiyohara H, Cyong JC, Yamada H. (1989). Relationship between structure and activity of the "ramified" region in anti-complementary pectic polysaccharides from *Angelica acutiloba* Kitagawa. *Carbohydrate Research* 193, 201-214.
- Leckie MJ, Ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, Mathur AK, Cowley HC, Chung KF, Djukanovic R, Hansel TT, Holgate ST, Sterk PJ, Barnes PJ. (2000). Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyperresponsiveness, and the late asthmatic response. *Lancet* 356, 2144-48.

- Maggi E, Parronchi P, Manetti R, Simonelli C, Piccinni MP, Rugin FS, De Carli M, Ricci M, Romagnani S. (1992). Reciprocal regulatory effect of IFN-gamma and IL-4 on the in vitro development of human Th1 and Th2 clones. *Journal of Immunology* 148, 2142-2147.
- Makare N, Bodhankar S, Rangari V. (2001). Immunomodulatory activity of alcoholic extract of *Mangifera indica* L. in mice. *Journal of Ethanopharmacology* 78, 133-137.
- Morita H, Nagashima S, Uchiumi Y, Kuroki O, Takeya K, Itokawa H, (1996). Cyclic peptides from higher plants. XXVIII. Antitumor activity and hepatic microsomal biotransformation of cyclic pentapeptides, astins, from *Aster tataricus*. *Pharmaceutical Bulletin* 44, 1026-1032.
- Nakajima H, Iwamoto I, Yoshida S. (1993). Aerosolized recombinant interferon-gamma prevents antigen-induced eosinophil recruitment in mouse trachea. *The American review of respiratory disease* 148, 1102-1104.
- Ooi VEC, Liu F. (2000). Immunomodulation and anticancer activity of polysaccharide protein complexes. *Currently Medicinal Chemistry* 7, 715-729.
- Patwardhan B, Kalbag D, Patki PS, Nagsampagi BA. (1990). Search of immunomodulatory agents: a review. *Indian Drugs* 28, 348-358.
- Ricci M, Matucci A, Rossi O. (1997). IL-4 as a key factor influencing the development of allergen-specific Th2-like cells in atopic individuals. *Journal of investigational allergology and clinical immunology* 7, 144-150.
- Romagnani S, Maggi E, Del Prete GF, Parronchi P, Tiri A, Ricci M. (1989). Role of interleukin 4 and gamma interferon in the regulation of human IgE synthesis: possible alterations in atopic patients. *International archives of allergy and applied immunology* 88, 111-113.
- Sanderson CJ. (1992). Interleukin-5, eosinophils and disease. *Blood* 79, 3101-3109.
- Shao Y, Ho C, Chin C. (1997). Aster lingulatosides C and D cytotoxic triterpenoid saponins from *Aster lingulatus*. *Phytochemistry* 44, 337-341.
- Shirota O, Morita H, Takeya K, Itokawa H, Iitaka Y. (1997). Cytotoxic triterpene from *Aster tataricus*. *Nature Medicine* 51, 170-172.
- Tepper R, Levinson DA, Stanger BZ, Campos-Tovinson J, Abbas AK, Leder P. (1990). IL-4 induces allergic-like inflammatory disease and alters T cell development in transgenic mice. *Cell* 62, 457-467.
- Van der Nat JM, 'tHart LA, Van der Sluis WG, Van Dijk H, Van den Berg AJJ, De Silva KTD, Labadie RP. (1989). Characterization of anti-complement compounds from *Azadirachta indica*. *Journal of Ethanopharmacology* 27, 15-24.
- Wang YZ. (2001). Thoughts on the Improvement of Chinese Medicine Decoction. *China Journal of Chinese Materia Medica*, 26, 134-136.
- Yamada H, Kiyohara H, Cyong JC, Kojima Y, Kumazawa Y, Otsuka Y. (1984). Studies on polysaccharides from *Angelica acutiloba*. *Planta Medica* 50, 163-167.
- Yamada H, Ohtani K, Kiyohara H, Cyong JC, Otsuka Y, Ueno Y, Omura S. (1985). Purification and chemical properties of anti-complementary polysaccharide from leaves of *Artemisia princeps*. *Planta Medica* 51, 121-125.
- Yamagishi T, Tsuboi T, Kikuchi K. (2003). Potent natural immunomodulator, rice water-soluble polysaccharide fractions with anticomplementary activity. *Cereal Chemistry* 80, 5-8.
- Yook CS, Rho YS, Sed SH, Leem JY, Han SH. (1996). Chemical components of *Acanthopanax divaricatus* and anticancer effect in leaves. *Yakhak Hoeji* 40, 251-161.