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SCREENING AND IDENTIFICATION OF POTENTIAL BIOACTIVE CONSTITUENTS IN A NEW HERBAL PRESCRIPTION SEAWEED COMPLEX PREPARATION

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Abstract

Background: Seaweed Complex Preparation (SCP) is a clinical traditional Chinese medicine preparation composed of seven traditional Chinese herbs, and has been used for treatment of lung cancer, liver cancer and digestive cancer.
Materials and Methods: In this study, the potential bioactive constituents of SCP were isolated and identified by chromatographic and spectroscopic methods. The immunomodulatory and DPPH radical scavenging activities of the constituents were also evaluated *in vitro*.
Results: Forty-three compounds were isolated from the EtOAc and *n*-BuOH fractions of the decocted extracts. More than 10 structural types of chemical constituents were found, including flavonoid glycosides, flavonoids, phenolic acid compounds, lignans, iridoid glycosides, and polysaccharides mainly composed of galactose and glucose. Flavonoid glycosides exhibited potential immunomodulatory by promoting splenic lymphocyte proliferation and stimulating the secretion of cytokines IL-2 and IFN-γ, as well as free radical (DPPH) scavenging activities. Caffeic acid derivatives showed remarkable free radical (DPPH) scavenging activity, while polysaccharides also displayed immunological enhancement effects.
Conclusion: Four molecular communities, flavonoid glycosides, flavonoids, phenolic compounds and polysaccharides, might be the potential bioactive components of SCP. Synergism of these four molecular communities in SCP may endue the anticancer properties and reflect the feature of multi-components and multi-targets of traditional Chinese medicine formula.
Keywords: Traditional Chinese Medicine Preparation, Seaweed Complex Preparation, Bioactive constituents, Free radical scavenging; Immunomodulatory

Introduction

Traditional Chinese Medicine Preparation (TCMP) is one of the main forms of Traditional Chinese medical practice and is usually composed of several herbs combined at an intrinsic mass ratio according to the compatibility theory and dialectical view. As a complementary medical system to western medicine, TCMP provides a unique theoretical and practical approach to the treatment of diseases over thousands of years in China (Tsai et al., 2014). The holistic and systematic ideas are essentially different from the thinking modes based on reductionism in western medicine. Generally, the curative effects of TCMP result from integrative contributions of a number of bioactive constituents, and this idea is consistent with the concept of modern system biology as well as network pharmacology. TCMP also has a creative character of synergistic and competitive action among bioactive components upon multiple target sites (Zhang et al., 2008). Although many TCMPs have proven to be effective by modern pharmacological studies and clinical trials, the bioactive constituents of most TCMPs remain mysterious and unclear. In fact, the components in a TCMP are usually complex mixtures, almost consisting of hundreds or even thousands of different chemical constituents but only a part of them may truly responsible for the pharmaceutical and toxic effects. Therefore, screening and analysis of the bioactive constituents in a TCMP are of primary importance both for its quality control and for elucidation of its therapeutic mechanisms.

Seaweed Complex Preparation (SCP) is a clinical traditional Chinese medicine preparation composed of seven herbs known as *Sargassum pallidum* (Turn.) C. Ag., radix of *Astragalus membranaceus* (Fisch.) Bge., fruit of *Ligustrum lucidum* Ait., rhizome of *Smilax glubra* Roxb., radix of *Dioscorea bulbifera* L., rhizome of *Clematis chinensis* Osbeck, and sclerotium of *Poria cocos* Wolf. As a clinical preparation, it has been used to treat lung cancer, liver cancer and gastric cancer, and also used to relieve the pain caused by chemotherapy (Liu et al., 2013). Our previous study found that SCP could significantly inhibit the growth of transplanted hepatic tumors of murine hepatocarcinoma cell line H22, and could also promote the proliferation of splenocytes and the phagocytosis of macrophages. In addition, SCP was found to have noticeable scavenging activities on DPPH and hydroxyl radicals (Liu et al., 2009). In present study, the bioactive components in SCP were isolated and identified with chromatographic and spectroscopic methods, and were screened and evaluated by using diverse bioassay models in an attempt to elucidate the effective chemical constituents and functional mechanism of SCP.

Materials and methods

General experimental procedures

^{1}H, ^{13}C NMR and 2D NMR spectra were recorded on a JEOLJNM-ECP600 MHz NMR spectrometer with TMS as an internal standard. ESI-MS spectra were obtained from a Micromass Q-TOF spectrometer. High performance liquid chromatography (HPLC) separation was performed on a Waters prep-HPLC system using a C_{18} (Kromasil, 5 μm, 250 × 10 mm) column coupled with a 2996 photodiode array detector. Silica gel (Qingdao Haiyang Chemical Group Co.; 200–300 mesh), Sephadex LH-20 (Amersham Biosciences) and octadecylsilyl silica gel (Unicorn; 45–60 μm) were used for column chromatography, and precoated silica gel

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plates (Qingdao Marine Chemical Factory, Qingdao, China; GF254) were used for thin layer chromatography.

Materials

The herbs, *S. pallidum* (Turn.) C. Ag., radix of *A. membranaceus* (Fisch.) Bge., fruit of *L. lucidum* Ait., rhizome of *S. glubra* Roxb., radix of *D. bulbifera* L., rhizome of *C. chinensis* Osbeck and sclerotium of *P. cocos* Wolf, were purchased from Qingdao Shandatianyuan Company, Shandong province, China, in June 2006 and identified by Prof. Feng-qin Zhou at Shandong University of Traditional Chinese Medicine (Jinan, China). All of the voucher specimens were deposited in the Key Laboratory of Marine Drugs, Ministry of Education, Ocean University of China (Qingdao, China).

1,1-diphenyl-2-picry-hydrazy (DPPH), butylated hydroxytoluene (BHT), and 3-(4,5-Dimethylthizol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma Chemical Co. (Saint Louis, Missouri, USA). RPMI-1640 medium was purchased from *Gibco*, Grand Island, NY. All of other chemicals and solvents were of analytical grade.

ICR mice (Grade II, 5 weeks old) weighing 18–22 g were purchased from the Center for New Drugs Evaluation of Shandong University (Certificate No. SKXK. 20030004, Jinan, China) and acclimatized for a week before used.

Extraction, isolation and purification of SCP decoction

The formula of SCP was composed of *S. pallidum* (450 g), *A. membranaceus* (450 g), *L. lucidum* (600 g), *S. glubra* (600 g), *D. bulbifera* (450 g), *C. chinensis* (600 g) and *P. cocos* (450 g). The powdered mixture of above herbs were decocted three times in boiling water (1:10, w/v, for 2 h; 1:8 w/v, 1.5 h; and 1:6, w/v, 1 h). The filtrate of the decoction was concentrated under vacuum to yield the SCP extract with a ratio of herb material to solution as 2:1 (g: ml), and stored at 4°C for later use. The extract was dissolved in water and partitioned successively with EtOAc and *n*-BuOH to yield EtOAc-fraction (35.0 g), *n*-BuOH-fraction (124.0 g), and water soluble fraction (872.0 g), respectively. The EtOAc and *n*-BuOH fractions were subjected to silica gel column chromatography, Sephadex LH-20 column chromatography and preparative HPLC to afford compounds **1**–**43**.

Composition analysis of water soluble fraction

The total sugar content was estimated by the phenol-sulfuric acid method using glucose as a standard (Dubois et al., 1956). The uronic acid content was determined by the carbazole–sulfuric acid method using glucuronic acid as a standard (Bitter and Muir, 1962). The monosaccharide composition of the polysaccharides in the water soluble fraction of SCP was determined using the acidic hydrolysate of the polysaccharides by reversed-phase HPLC after pre-column derivatization and UV detection with minor modifications (Liu et al., 2015). A polysaccharide sample (5 mg) was hydrolyzed with 2 mol/l trifluoroacetic acid at 100°C for 6 h. Excessive acid was removed by co-distillation with methanol after hydrolysis. The dry hydrolysate (1 mg) was dissolved in 100 µl 0.3 mol/l NaOH, and added to 120 µl 0.5 mol/l methanol solution of 1-phenyl-3-methyl-5-pyrazolone (PMP) at 70°C for 1 h. The mixture was added to 100 µl 0.3 mol/l HCl, vigorously shaken, and then centrifuged at 2400 *g* for 5 min. The supernatant containing the labeled carbohydrate was filtered through 0.22 µm nylon membranes. The filtrate (10 µl) was analyzed on a HPLC system (Aglient 1260 Infinity HPLC with a C_{18} column (Kromasil, 4.6×250 mm, particle size 5 µm) at 30°C using a mixed mobile phase of 0.1 mol/l KH_2PO_4 (pH 10)–acetonitrile (77:23) at a flow rate of 1.0 ml/min. Monosaccharides were identified by comparison with the authoritative monosaccharides including mannose, gluconsamine, glucuronic acid, galacturonic acid, glucose, galactose, arabinose and fucose.

Splenic lymphocyte proliferative activity assay

Male mice in clean grade were killed by cervical dislocation and spleens were removed aseptically. Erythrocytes were depleted with ammonium chloride buffer solution. Lymphocytes were washed twice with PBS containing 5% FBS and final splenic lymphocyte suspension at a concentration of 5×10^6 cells/ml of RPMI 1640 medium supplemented with 12 mM HEPES (4-(2-hydroxyerhyl) piperazine-1-erhanesulfonicacid, pH 7.1), 0.05 mM 2-mercaptoethanol, 100 IU/ml penicillin, 100 µg/ml streptomycin, and 10% FBS.

The above prepared splenic lymphocyte solution (100 µl) was placed in a 96-well flat-bottom plate. The tested compound (1 µg/ml, 10 µg/ml, and 100 µg/ml, respectively) was added to the cell suspension, and RPMI1640 medium were added to give a final volume of 200 µl. The control was added same volume of RPIM-1640 solution. The mixture was incubated at 37°C with 5% CO_2 for 48 h, and 20 µl of the solution containing 5 mg/ml of MTT was added into each well and incubated for 4 h. Subsequently, the plates were centrifuged (1400×g, 5 min) and the untransformed MTT was removed by pipetting. DMSO (200 µl) solution (192 µl DMSO with 8 µl 1M HCl) was added to each well, and the plates were mixed. The absorbance at 570 nm was evaluated using the enzyme-linked immunosorbent assay (Xu et al., 2009). The proliferation rate was calculated using the following equation:

Proliferation rate (%) = $[(A_1-A_0)/ A_0\times100]$

Where A_0 is the absorbance of the control reaction and A_1 is the absorbance in the presence of the sample. The values were presented as mean ± S.E. (n = 3).

Cytokine levels in the supernatants of cultured splenocytes

Levels of interleukin-2 (IL-2) and interferon-γ (IFN-γ) produced by splenocytes were measured as described in the previous literature with some modifications (Han et al., 2010). Splenocytes (5×10^5 cells/well) from male mice were prepared as previously described. The tested compound (1 µg/ml, 10 µg/ml, and 100 µg/ml, respectively) was added to the cell suspension. After 48 h, the plate was centrifuged at 1400×g for 5 min and the supernatant was collected for the detection of IL-2 and IFN-γ levels using commercial ELISA kits according to the manufacturer's instruction.

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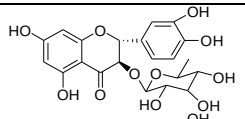
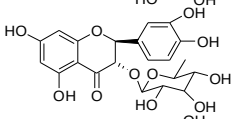
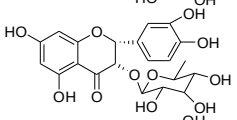
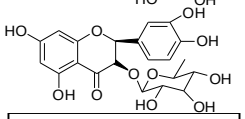
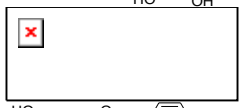
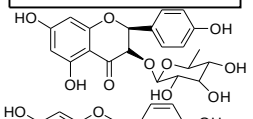
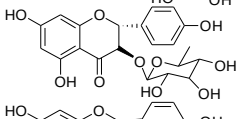
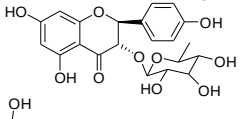
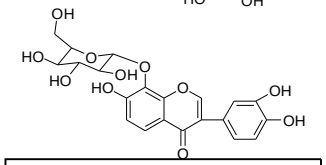
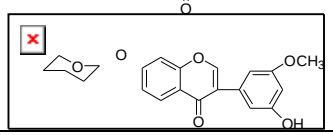
Table 1: Compounds isolated from SCP and their structures and bioactivities

Compd	Name	Structure	Structural type	Main bioactivity
1	astilbin (80.0 mg) (Yuan et al., 2004)		flavonoid glycoside	DPPH radical scavenging activity [b] Immunomodulatory activity [c]
2	seoastilbin (15.0 mg) (Yuan et al., 2004)		flavonoid glycoside	DPPH radical scavenging activity [b] Immunomodulatory activity [b]
3	isoastilbin (18.0 mg) (Yuan et al., 2004)		flavonoid glycoside	DPPH radical scavenging activity [a] Immunomodulatory activity [b]
4	neoisoastilbin (12.0 mg) (Yuan et al., 2004)		flavonoid glycoside	– [d]
5	engeletin (5.0 mg) (Dulce et al., 1997)		flavonoid glycoside	– [d]
6	neoengeletin (7.0 mg) (Dulce et al., 1997)		flavonoid glycoside	DPPH radical scavenging activity [b]
7	isoengeletin (3.0 mg) (Gaffield et al., 1975)		flavonoid glycoside	– [d]
8	neoisoengeletin (15.0 mg) (Gaffield et al., 1975)		flavonoid glycoside	– [d]
9	7,3',4'-trihydroxyisoflavone-8-O-*β*-D-glucoside (2.0 mg) (Rong et al., 1998)		flavonoid glycoside	– [d]
10	3'-methoxy-5'-hydroxyisoflavone-7-O-*β*-D-glucoside (15.0 mg) (Cao et al., 1999)		flavonoid glycoside	– [d]

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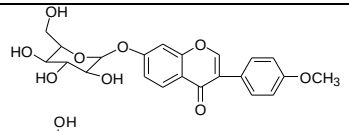
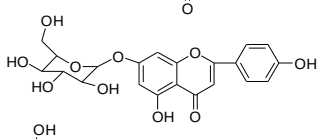
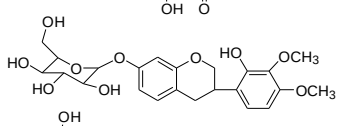
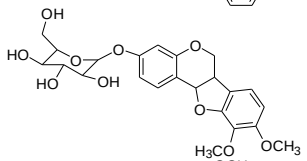
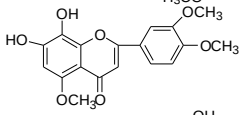
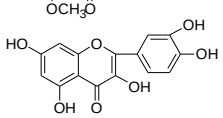
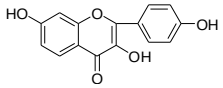
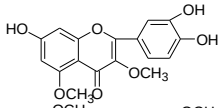
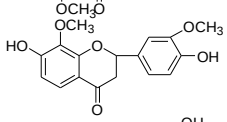
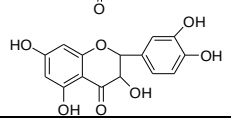
11	ononin (20.0 mg) (Li et al., 2004)		flavonoid glycoside	Immunomodulatory activity [b]
12	genistin (7.0 mg) (Li et al., 2009)		flavonoid glycoside	– [d]
13	7,2'-dihydroxy-3',4'-dimethoxyisoflavaone-7-O -*β*-D-glucoside (15.0 mg) (Ma et al., 2005)		flavonoid glycoside	– [d]
14	9,10-dimethoxypterocarpan-3-O-*β*-D-glucoside (7.0 mg) (Li et al., 2004)		flavonoid glycoside	– [d]
15	5,3'-dimethoxy-7,8,4'-trihydroxyflavone (3.0 mg) (Miwa et al., 1986)		flavonoid	– [d]
16	quercetin (4.0 mg) (Zhang et al., 2007)		flavonoid	DPPH radical scavenging activity [a]
17	resokaempferol (2.5 mg) (Pan et al., 2006)		flavonoid	– [d]
18	caryatin (2.5 mg) (Gao et al., 2001)		flavonoid	DPPH radical scavenging activity [b]
19	7,4'-dihydroxy-8,3'-dimethoxyflavanone (3.4 mg) (Cleofe and John, 1998)		flavonoid	– [d]
20	taxifolin (5.0 mg) (Li et al., 1999)		flavonoid	– [d]

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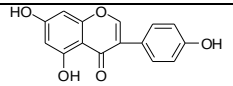
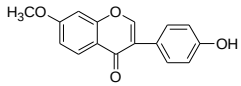
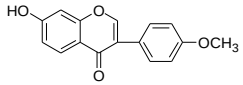
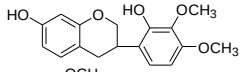
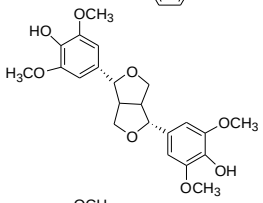
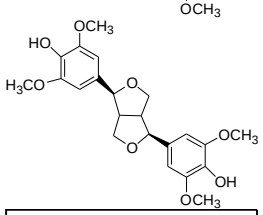
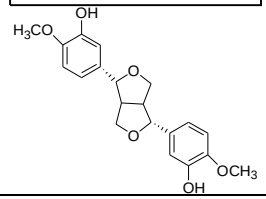
21	genistein (2.5 mg) (Li, Zhou, et al., 2007)		flavonoid	– [d]
22	4’-hydroxy-7-methoxyisoflavone (3.0 mg) (Li et al., 2001)		flavonoid	– [d]
23	formononetin (2.0 mg) (Li et al., 2003)		flavonoid	– [d]
24	7,2’-dihydroxy-3’,4’-dimethoxyisoflavan (7.5 mg) (Zhao et al., 2002)		flavonoid	– [d]
25	(+)-syringaresinol (2.0 mg) (Guo et al., 2006)		lignan	– [d]
26	(-)-syringaresinol (2.0 mg) (Zhang and Sun, 2006)		lignan	– [d]
27	clemaphenol A (3.0 mg) (He, Zhang, et al, 2001)		lignan	– [d]
28	Pinoresinol (3.5 mg) (He, Zhang, et al, 2001)		lignan	– [d]

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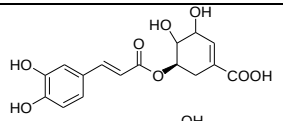
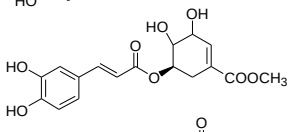
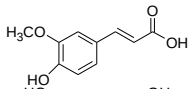
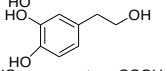
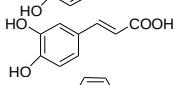
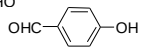
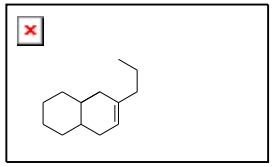
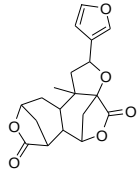
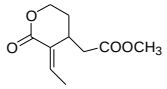
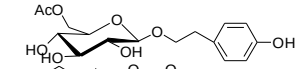
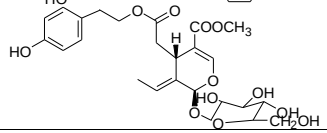
29	5-O-caffeoylshikimic acid (20.0 mg) (Markus et al., 1992)		phenolic acid	DPPH radical scavenging activity [a]
30	dactylifric acid (7.0 mg) (Maier et al., 1964)		phenolic acid	DPPH radical scavenging activity [a]
31	ferulic acid (5.0 mg) (Zheng et al., 2004)		phenolic acid	– [d]
32	4-dihydroxyphenylethanol (2.5 mg) (Zhang et al., 2006)		phenolic acid	DPPH radical scavenging activity [b]
33	caffeic acid (5.0 mg) (Zheng and Hu, 2006)		phenolic acid	DPPH radical scavenging activity [a]
34	p-hydroxybenzaduhyde (2.0 mg) (Lai et al., 2009)		phenolic acid	– [d]
35	20-hydroxyecdysone (5.0 mg) (Lin et al., 2006)		steroid	– [d]
36	diosbulbin-B (250.0 mg) (Liu et al., 2004)		diterpene	– [d]
37	(3-Ethylidene-2-oxo-tetrahydro-pyran-4-yl)-acetic acid methyl ester (5.0 mg) (Liu et al., 2010)		monoterpene	– [d]
38	*β*-D-glucopyranoside, 2-(4-hydroxyphenyl), 6-acetate (25.0 mg) (Yu et al., 2008)		phenolic glycoside	– [d]
39	nuezhenoside (10.0 mg) (Li, Zu et al., 2007)		iridoid glycoside	– [d]

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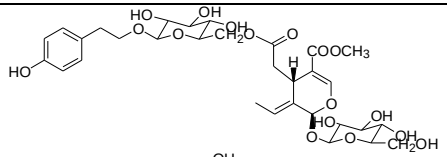
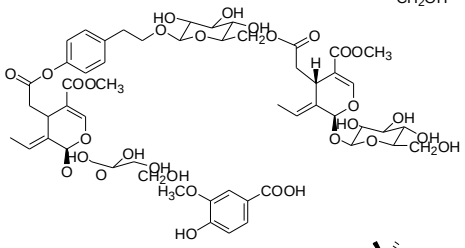
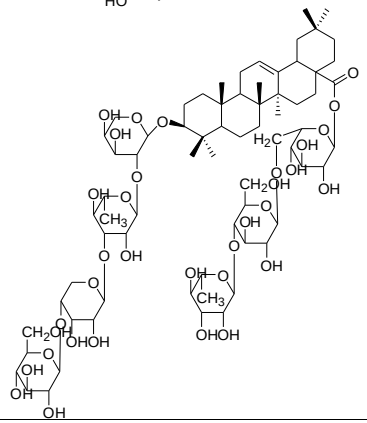
40	ligustroside (15.0 mg) (He, Dong et al., 2001)		iridoid glycoside	– [d]
41	nuezhenoside G13 (50.0 mg) (Li, Zu et al., 2007)		iridoid glycoside	– [d]
42	vinillic acid (6.0 mg) (Li, Zhang et al., 2007)		phenolic acid	– [d]
43	clematichinenoside C (12.0 mg) (Shao et al., 1996)		steroid saponin	– [d]

a, strong; b, moderate; c, weak; d, no activity has been found.

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DPPH radical scavenging activity

The free radical (DPPH) scavenging activity was determined by the method described in a previous report (Fu et al., 2009). The tested compound (1 ml) at the concentration of 200 µg/ml was added to 4 ml of 0.004% methanol solution of DPPH. After 30-min incubation in the dark at room temperature, the absorbance was measured at 517 nm using a spectrophotometer. A lower absorbance of the reaction mixture indicates a higher free radical scavenging activity. BHT was used as a positive control. The capability to scavenge the DPPH radical was calculated using the following equation:

Scavenging rate (%) = $[(A_0-A_1)/ A_0\times100]$
Where A_0 is the absorbance of the control reaction (containing all reagents except the samples) and A_1 is the absorbance in the presence of the sample. The values were presented as means±S.E. (n = 3).

Statistical analysis

Data were expressed as mean standard deviation (SD) of three replicated determinations. Analysis of variance (ANOVA) was carried out using the software Microcal Origin 6.0 (Microcal Software, Inc., Northampton, USA). Mean values were compared using the Turkey test. $P < 0.05$ was considered statistically significant.

Results

Chemical constituents of the EtOAc and n-BuOH fractions from SCP

Forty-three compounds were isolated from the EtOAc and *n*-BuOH fractions of SCP by column chromatography on silica gel, Sephadex LH-20, ODS, and semi-preparative HPLC. Their structures were elucidated using comprehensive spectroscopic methods including 1D and 2D NMR, MS, UV and IR, combined with a variety of chemical methods, and by comparison their data with those reported previously in the literature. The isolated compounds were involved in ten types of chemical structures, including fourteen flavonoid glycosides (**1**–**14**), ten flavonoids (**15**–**24**), four lignans (**25**–**28**), seven phenolic acid compounds (**29**–**34, 42**), one steroid (**35**), one diterpene (**36**), one monoterpene (**37**), one phenolic glycoside (**38**), three iridoid glycosides (**39**–**41**) and one steroid saponin (**43**) (Table 1). Among the isolated compounds, about of 50% of them were flavonoid glycosides and flavonoids, and it could be concluded that flavonoid glycosides, flavonoids, phenolic compounds and lignans were the major structure types in the EtOAc and *n*-BuOH fractions of SCP.

Composition analysis of water fraction from SCP

The polysaccharide and uronic acid content in the water fraction of SCP was 56.90% and 6.32%, respectively. The monosaccharide composition analysis of the SCP water fraction is shown in Fig. 1. HPLC analysis demonstrated that the SCP polysaccharides in water fraction were mainly composed of galactose and glucose with small amounts of mannose, galacturonic acid and arabinose.

Splenic lymphocyte proliferative activity of the isolated compounds

The immune system plays an important role in antitumor defense. Splenic lymphocyte proliferation is a crucial event in the activation cascade of both cellular and humoral immune responses. Many reports suggested that antitumor activities of natural medicines were mediated through enhancing the immune responses (Huang et al., 2013). Therefore, in this study, the effects of the isolated compounds from SCP on splenic lymphocyte proliferation were determined. Compounds **1**, **2**, **3**, **4** and **11** were found to enhance splenic lymphocyte proliferation compared with the control at a concentration of 1 µg/ml with the rates of 9.67±0.45%, 6.50±0.93%, 19.02±1.67%, 21.46±1.15%, and 6.93±1.98%, respectively. At the concentration of 100 µg/ml, the proliferation rates of compounds **2** and **11** reached 19.86±1.21% and 28.09±1.95%, respectively. It could be proposed that the flavonoid glycosides in SCP might possess moderate lymphocyte proliferative activity. Interestingly, the water fraction of SCP could also promote the proliferation of splenic lymphocytes compared with the untreated cells with the rate of 55.32±0.32%. The lymphocyte proliferative activity of the water fraction might be associated with the polysaccharides.

Cytokine levels in the supernatants of cultured splenocytes treated with the isolated compounds

Knowing that cytokines play a prominent role in the development of immune response, we investigated the effects of the isolated compounds as well as the water fraction of SCP on the secretion of cytokines IL-2 and IFN-γ produced by splenocytes. Compounds **3**, **4** and **11** were observed to augment the production of IL-2 and IFN-γ from splenocytes in varying degrees compared with the cell control. In single stimulation with compounds **3** and **4** at 10 µg/ml, the concentrations of IL-2 were up to 32.98 pg/ml and 54.64 pg/ml, respectively, significantly higher than that of cell control group (Fig. 2). Correspondingly, the productions of IFN-γ by compounds **3** and **4** at 10 µg/ml was significantly higher than that of cell control group, with the secretion of IFN-γ being 28.91 pg/ml and 56.72 pg/ml, respectively. In addition, compound **11** could also significantly increase the production of IL-2 and IFN-γ at 100 µg/ml, with the secretion of 23.21 pg/ml and 46.51 pg/ml, respectively. These results indicated that the flavonoid glycosides in SCP might be the bioactive components that could improve the cellular and humoral immune responses. The water fraction of SCP also showed higher immunomodulatory activity compared with the control cell, improving the production of IL-2 and IFN-γ at 100 µg/ml with the secretion up to 39.17 pg/ml and 54.42 pg/ml, respectively.

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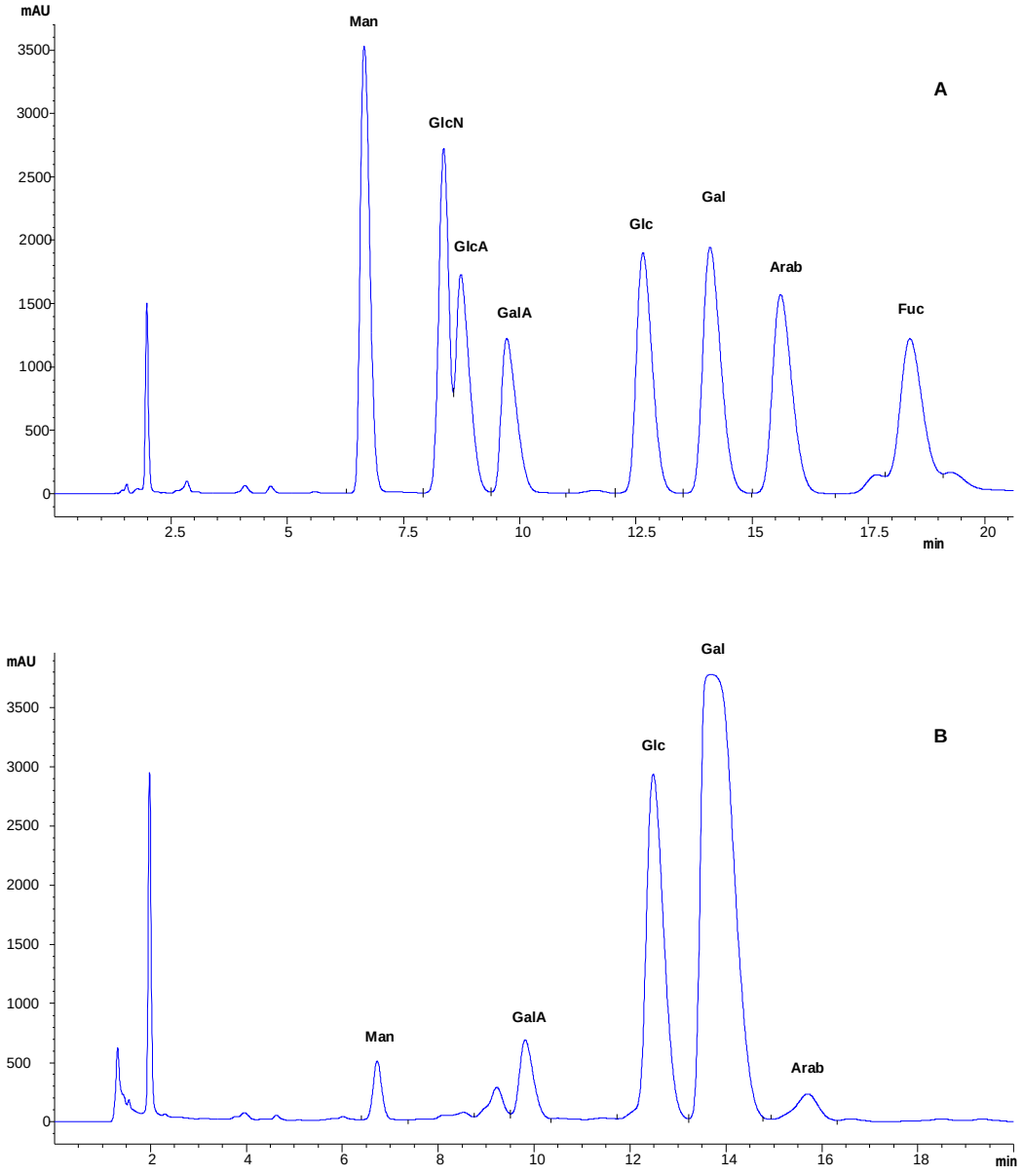

Figure 1: HPLC analysis of the monosaccharide composition in the SCP water fraction. The standards were separated under the conditions described in A, and the derivatives of SCP water fraction hydrolysate were separated as described in B (Man, mannose; GlcN, glucosamine; GlcA, glucuronic acid; GalA, galacturonic acid; Glc, glucose; Gal, galactose; Arab, arabinose; Fuc, fucose).

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DPPH radical scavenging activity of the isolated compounds

The DPPH radical scavenging activity is often used to evaluate the capacity of antioxidant compounds. The DPPH scavenging activities of the compounds isolated from the EtOAc and *n*-BuOH extract of SCP are shown in Fig. 3. The scavenging rate of 11 compounds was beyond 50% at 200 μg/ml. Compounds **3**, **4**, **16**, **29**, **30** and **33** exhibited notable DPPH radical scavenging activity, which is higher than that of the positive control BHT. As a result, flavonoids and caffeic acid derivatives in SCP possessed excellent DPPH radical scavenging activities.

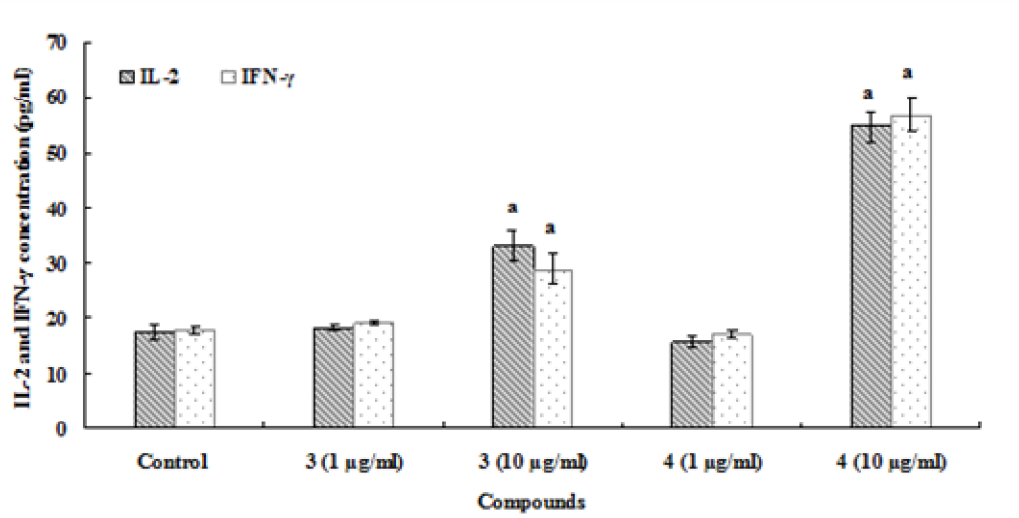

Figure 2: Effects of IL-2 and IFN-γ secretion in splenocytes in stimulation of compounds **3** and **4** (The results were expressed as mean values ± SD, 'a' indicates the significant difference at the level of 0.05 compared with control).

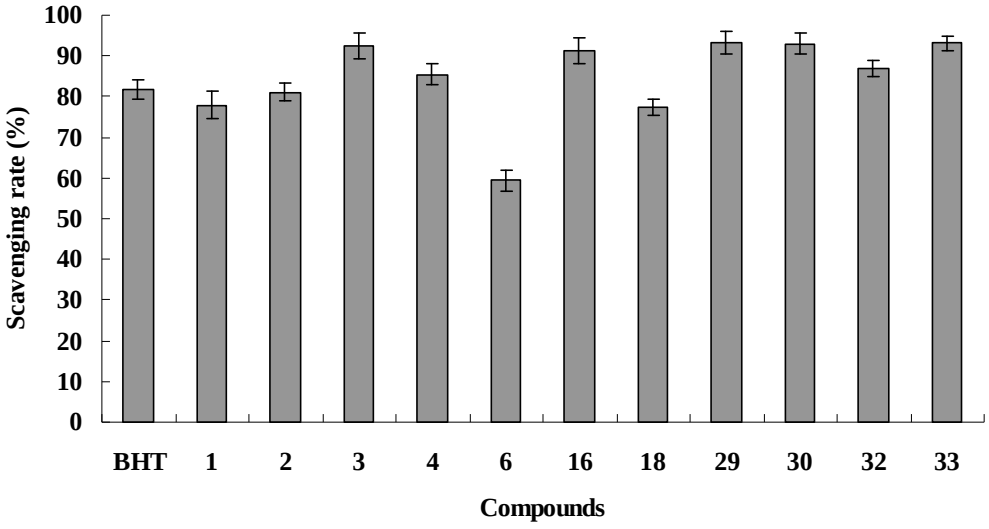

Figure 3: DPPH radical scavenging activity of part of the compounds isolated from EtOAc and *n*-BuOH extract of SCP at the concentration of 200 μg/ml.

Discussion

SCP was an organic unity of complicated chemical constituents with multiple antitumor approaches. The results of this study

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suggested that SCP comprised several principal components, including flavonoids, flavonoid glycosides, phenolic compounds and polysaccharides. Each component might exert a different function. It was necessary to assess the pharmacodynamic function of each component in the medication.

There exists a close relationship between the occurrence, growth and decline of tumor and immune states. The low immune function of an organism may not only result in the generation and development of a tumor but is an important factor that prevents the tumor patients from recovering. Immunomodulation with natural or synthetic substances may be considered as an alternative manner for the prevention and cure of neoplastic diseases (Xu et al., 2009). Cytokine production by $CD4^+$ helper T lymphytocytes during immune response plays an important role in regulating the nature of the response. For example, IFN-γ and IL-2 are secreted by type 1 helper T cells (Th1 cells) and mediate cellular immunity. They both can promote the proliferation and differentiation of Th1 cells (Lichterfield et al., 2004). IFN-γ is a pleiotropic cytokine with immunomodulatory effects on different kinds of immune cells. While IL-2 is one of the cytokines that plays an important role in immunomodulation and a critical component in activation and differentiation of T lymphocytes as well as the regulation of the immune system (Huang et al., 2013). Therefore, preliminary assessment for activation extent of T cells could be made by detecting the contents of IFN-γ and IL-2. Clinical studies have shown that the immune system of tumor patients is in a disordered state where the IL-2 level is declined obviously and Th2 cells are in a relatively dominant position, while Th1 cells that are believed to act as an antitumor specific immune response are inhibited (Yu et al., 2011). In present study, the flavonoids and polysaccharides in SCP not only significantly promoted the proliferation of splenic lymphocytes, but also increased the concentration of IFN-γ and IL-2 secreted by T lymphocytes. These bioactive constituents could promote the expression of Th1 cytokines, and improve cell immune function to resist cancer. To sum up, the flavonoid glycosides and polysaccharides in SCP could enhance the host immunity, which is recognized as a possible means of inhibiting tumor growth without harming the host.

Cancer formation can be directly induced by free radicals thereby the radical scavenging activity is one of the important functional properties for bioactive compounds. The DPPH radical scavenging activity was often used to evaluate the capacity of antioxidant compounds. Flavonoids and caffeic acid derivatives in SCP showed strong antioxidant activities in present study.

In conclusion, four molecular communities in SCP, including flavonoid glycosides, flavonoids, phenolic compounds and polysaccharides, might be involved in antitumor effects. As shown in Fig. 4, flavonoid glycosides and polysaccharides exhibited immunomologcial enhancement effects, while flavonoid glycosides, flavonoids and caffeic acid derivatives showed remarkable antioxidant activities. Synergism of these four molecular communities, together with the auxiliary effects of other components in SCP, may procure the anticancer properties and embody the feature of multi-components and multi-targets of traditional Chinese medicine preparations.

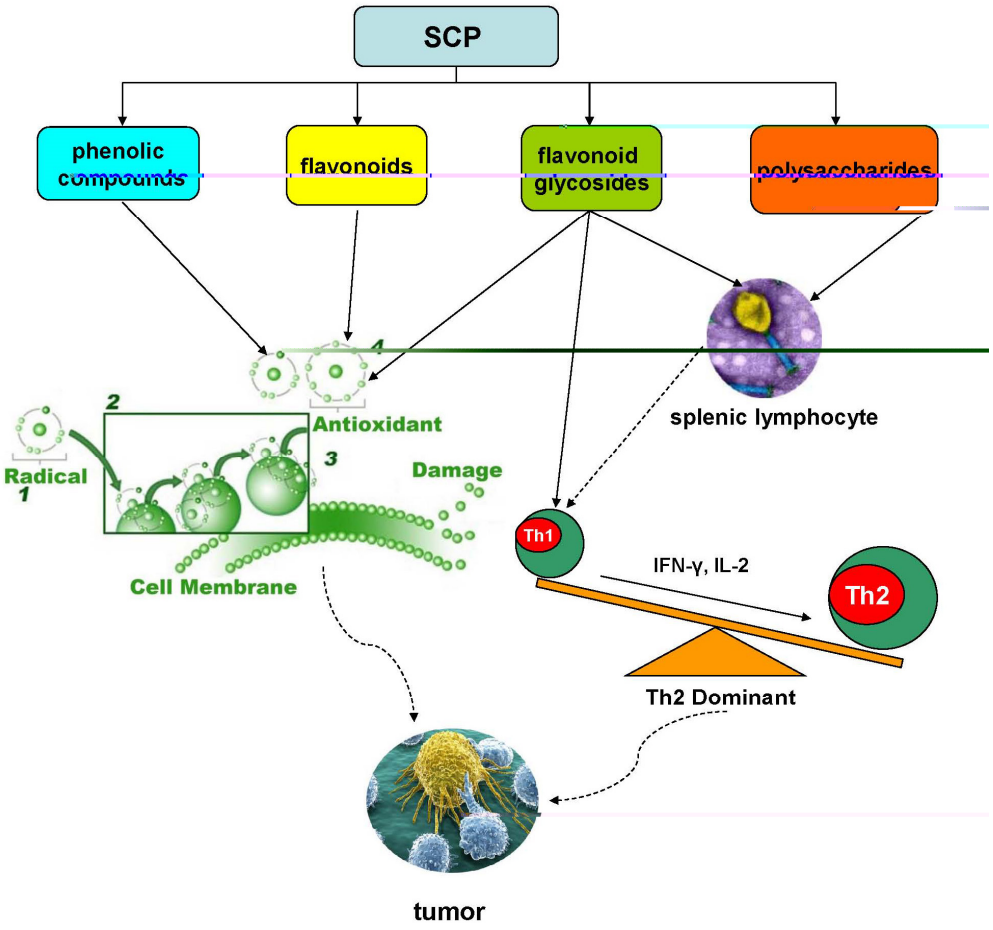

Figure 4: Schematic diagram of the antitumor effects of four molecular communities in SCP

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