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Research Article
Immunostimulatory and Anticancer Activities of Polysaccharides Extracted from Traditional Anticancer Chinese Medicinal Herbs

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Abstract

Background and Objective: It is evident from the scientific literature that plant and mushroom polysaccharides possess significant antioxidant, immunomodulatory and anticancer activities. Sixteen traditional Chinese anticancer herbs were evaluated for the immunomodulatory and anticancer potential of their polysaccharides. This study also aimed to correlate the bioactivities of these polysaccharides with their monosaccharide composition and to identify the best herbs for further detailed studies. **Methods:** Polysaccharides were extracted from the selected traditional Chinese medicinal (TCM) herbs and their biological activities examined. The antioxidant activities were examined using DPPH$^{\bullet}$ scavenging, ABTS$^{\bullet+}$ scavenging and iron chelating assays. The immunomodulatory properties of the polysaccharides were determined by evaluating their capacity to activate mouse macrophages (RAW 264.7) to produce the cytokines, namely, interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α). The anticancer activities of the polysaccharides were determined against five human cancer cell lines. Cell viabilities were evaluated by the MTT test to assess the toxicities of these polysaccharides. The total sugar content and the monosaccharide composition of the polysaccharides were determined with a view to correlate their bioactivities with chemical constituents. All data were analyzed using ANOVA and Duncan's multiple range methods. **Results:** The polysaccharides isolated from *Artemisia annua* L., *Lobelia chinensis* Lour, *Amauroderma rugosum* (*Blume* and *T. nees*), *Artemisia scoparia* Waldst. and *Kit*, *Artemisia vulgaris* L., *Curcuma aromatic* Salisb, *Rheum palmatum* L. and *Cyperusrotundus Blanco* showed significant anticancer, immunomodulatory and antioxidant activities with low toxicity. The results suggested that the polysaccharides extracted from *A. annua*, *L. chinensis*, *A. rugosum* and *S. suberectus* have strong potential for immunotherapy and hence suitable candidates for cancer treatment. **Conclusion:** Polysaccharides from several herbs were found to exhibit significant antioxidant, immunostimulatory and anticancer activities. The results demonstrated that, *A. annua*, *L. chinensis* and *A. rugosum* and *S. suberectus* are highly suitable herbs for the discovery of anticancer polysaccharides.

Key words: Chinese medicinal plants, antioxidant activities, immunomodulatory properties, anticancer, polysaccharides

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Traditional Chinese medicinal (TCM) herbs have a long history of successfully treating various life-threatening diseases including cancer[1-3]. Numerous scientific studies involving TCM plants exist in the literature[2-18] and some of these have led to the discovery of several important lead compounds of therapeutic value and a few are in the advanced stage of clinical usage/trials. In this context plant/mushroom polysaccharides are of great interest in order to discover novel therapeutic agents with minimal side effects[5,15,19]. In the past several years, botanical polysaccharides with immunomodulatory and anti-proliferative properties have been the focus of attention for the discovery of chemo-immunotherapeutic agents and significant progress has been achieved in this field[5,10,11,15,20]. For example, lentinanderived from *Lentinula edodes*[20,21], polysaccharide Krestin (PSK) derived from *Coriolus versicolor*[10,21], polysaccharopeptide(PSP) isolated from *Coriolus versicolor*[10,20,22] and schizophyllan from *Schizophyllum commune*[20,23] are important anticancer agents.

A program of research has been initiated in Authors' laboratory for the discovery of novel anticancer polysaccharides from a selected set of herbs that have a wealth of traditional knowledge for the treatment of cancer[5,10,11,15,20,22]. Selection of the herbs for this study was based on the available traditional knowledge on their anticancer and immunomodulatory properties[2,24-38]. These properties for the sixteen herbs studied in this study were summarised in Table 1. It is important to note that the polysaccharides from most of these herbs are yet to be systematically examined using contemporary scientific tools[32].

TCM practitioners commonly use hot water extracts for the treatment of cancer and other diseases[2]. Abundant traditional knowledge on the selected herbs and the limited scientific understanding of their polysaccharides warrant further study. Therefore, this study aims to extract water-soluble polysaccharides and examine their immunostimulatory and anticancer properties. Major significance of this research was to determine the best herbs that contain novel polysaccharides with immune-enhancing and anticancer properties that will ultimately lead to the discovery of immuno-chemotherapeutic agents. Cancer therapy is expensive and the patients from many developing countries cannot afford the cost. Hence, the discovery of novel therapeutics from the medicinal herbs will provide great benefit to humanity.

The objectives of this study were to determine antioxidant, immunostimulatory and anticancer properties of herbal polysaccharides. The results are expected to identify the herbs containing polysaccharides with good immuno-chemotherapeutic value. Outcomes of this study will open the way for bioactivity-guided isolation of polysaccharides from anticancer TCM herbs.

MATERIALS AND METHODS

This study was carried out between March, 2014 to November, 2016 as part of the program for the discovery of novel anticancer agents in Authors' Laboratory.

Table 1: Anticancer TCM herbs used in this research and their properties

Plant number	Name of herbs	Traditional uses and biological activities	References
P1	*Akebia quinata (Houtt.) Decne.*	Treatment of rheumatism, allergies diabetics and anti-cancer (sarcoma)	Kang *et al.*[24]
P2	*Alpinae officinarum Hance*	Anticancer and anti-allergic	Samarghandian *et al.*[25]
P3	*Artemisia annua* L.	Treat malaria and cancer	Chu *et al.*[26]
P4	*Artemisia scoparia Waldst.* and *Kit.*	Antioxidant, treat malaria and cancer	Huang *et al.*[2]
P5	*Artemisia vulgaris* L.	Anti-cancer, inhibition growth of HL-60 leukemic cell line by mitochondria-dependent apoptosis	Saleh *et al.*[27]
P6	*Citrus reticulate Blanco*	Anti-cancer, inhibition growth of Human Gastric Cancer Cells SNU-668	Kim *et al.*[28]
P7	*Curcuma aromatic Salisb*	Anti-tumour, inhibition growth of lung carcinoma cells	Ma *et al.*[29]
P8	*Cynanchum paniculatum* L.	Anti-cancer	Kim *et al.*[30]
P9	*Cyperusrotundus* L.	Antidiabetic, anti-obese, anti-platelet, anti-allergic, anti-inflammatory and anticancer	Peerzada *et al.*[31]
P10	*Lobelia chinensis Lour*	Anti-mutagenic activity and Anti-microbial activity and anticancer,	Li *et al.*[32]
P11	*Polygonum cuspidatum Sieb. et Zucc*	Anticancer, inhibition growth of human skin melanoma cells	Lee *et al.*[33]
P12	*Rabdosia rubescens (Hamst.)Wuet.*	Anticancer, apoptosis in human laryngeal cancer cells	Kang *et al.*[34]
P13	*Rheum palmatum* L.	Antitumor, anti-inflammatory, antimicrobial and hemostatic properties	You *et al.*[35]
P14	*Spatholobus suberectus Dunn.*	Anticancer	Wang *et al.*[36]
P15	*Xanthium sibiricum* L.	Anti-cancer, anti-inflammatory responses via the inhibition of nuclear factor-κB (NF-κB) and signal transducer and activator of transcription 3 (STAT3) in murine macrophages	Ju *et al.*[37]
P16	*Amauroderma rugosum* (*Blume* and *T. Nees*)	Anti-cancer	Chan *et al.*[38]

Procurement of medicinal plants associated with this research: Sixteen herbal plant materials were purchased from a Chinese herbal medical centre known as Beijing Tong Ren Tang located in Sydney (Australia). Sample specimen of all the herbs is stored in our research laboratory. This company has branches all over the world and is renowned for their best practice in TCM. The herbs traded in Sydney centre have approval from both the Australian and Chinese Governments. Scientific names of the herbs studied in this research are listed in Table 1.

Chemicals and materials: The 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), dimethyl sulfoxide (DMSO), ferrozine, 95% ethanol, ascorbic acid, sulfanilamide, N-(1-1-napthyl) ethylenediaminedihydrochloride, lipopolysaccharide (LPS)were purchased from Sigma (Australia) and Lomb Scientific Pty Ltd (Australia). The foetal bovine serum (FBS), antibiotics and Dulbecco's modified Eagle's medium (DMEM) with gluMax were purchased from BD Bioscience (USA). The tumour necrosis factor-α (TNF-α) and interleukin (IL-6) (mouse)-ELISA standards and antibodies were purchased from BD Bioscience (USA). Mouse macrophage cells (RAW 264.7) were purchased from Sigma-Aldrich. Five tumour cell lines used in this study, namely, MCF7 (ATCC HTB-22 breast carcinoma), HT29 (ATCC HTB-38 colon carcinoma), A549 (ATCC CCL-185 lung carcinoma), HepG2 (ATCC HB-8065 hepatocytes carcinoma) and MiaPAca2 (ATCC CRL-1420 pancreatic cancer) were purchased from the American Type Culture Collection.

Extraction of crude polysaccharides from medicinal herbs: 20-40 g of dried plant samples were ground to powder form and mixed well. The powdered material was subjected to hot water extraction using the autoclave method (at 121°C for 2 h) and cooled to laboratory temperature before separating the supernatant by filtration. The supernatant (extract) was then treated with 95% ethanol (extract: ethanol = 1:4 v/v) for 24 h at 2.1°C. The entire extraction process is illustrated in Fig. 1. The dry polysaccharide extracts were dissolved in deionised water (10 mg mL^{-1}) and mixed with 1/5 volume mixture of Sevag reagent to remove the free protein[15]. The de-proteinated polysaccharide extracts were stored at -20°Cuntil further use.

Analysis of chemical composition: The method developed originally by DuBois *et al.*[39] and Zhang *et al.*[15] was employed to measure the total sugar content. Glucose was used to build a standard curve:

$$y = 0.0018x+0.0374 (R^2 = 0.9964)$$

The method of Lowry *et al.*[40] was employed to measure the total bound protein in the polysaccharide samples.

Gas chromatographic analysis was performed to measure the mono-sugar content of the polysaccharides. The analysis was performed using a Hewlett Packard 7890B gas chromatograph with a FID detector and a capillary polar column (HP-5 column). The method developed by Jones and Albersheim[41] and Zhang *et al.*[15] was used to prepare the polysaccharide samples and for GC analysis. Rhamnose, ribose, fucose, arabinose, xylose, mannose, galactose and glucose were used as standards.

Bio-assays

Antioxidant activities

Scavenging activity against DPPH·radicals: The Blois method[12,42,43] was employed to determine the DPPH· scavenging ability of polysaccharide samples. Ascorbic acid was employed as positive control and deionised water as blank. The absorbance values were determined using UV spectrophotometer at 492 nm (Multiskan 141 EX, Thermo Electron, USA). Regression of the data gave a linear standard curve (with R^2 = 0.9715) represented by the following equation:

$$Y = -0.0026X+0.5578$$

DPPH· scavenging potential of the polysaccharides was determined as the ascorbic acid equivalence using the above equation.

ABTS·+radical scavenging assay: The methodology employed for this assay was similar to that published in the literature[17,43]. Ascorbic acid was employed as positive control with PBS buffer (pH 7.4) as blank. A standard curve was built using different concentrations of ascorbic acid solution (prepared in 60% methanol) in the range of 0-400 μM. Absorbance values were determined using a UV spectrophotometer at 734 nm[17,28] (Multiskan 141 EX, Thermo Electron, USA).

Regression of the data gave a linear standard curve (with R^2 = 0.9852) represented by the following equation:

$$Y = -0.0019X+0.7274$$

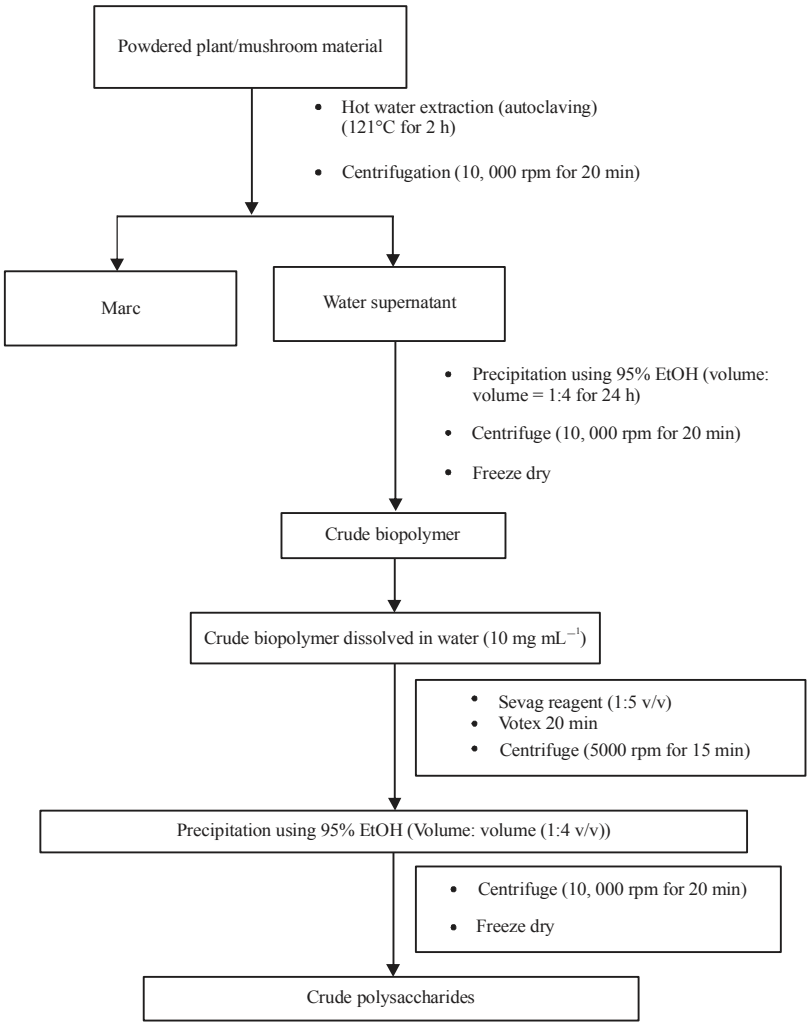

Fig. 1: Flow chart for the extraction of polysaccharides from selected herbs

The $ABTS^{\bullet+}$ scavenging capacities of the polysaccharides were determined as the ascorbic acid equivalence using the above equation.

Fe^{2+}chelating assay: The affinity of polysaccharides to complex with Fe^{2+} was determined by measuring the absorbance of the complex formed in the presence of ferrozine[18,44]. First, 0.1 mL of polysaccharide sample was mixed with 0.5 mL $FeCl_2$ (0.2 mM) to form the Fe-polysaccharide complex. To this complex, 0.2 mL of ferrozine (5 mM) was added and thoroughly mixed to trigger the competition between polysaccharide and ferrozine for Fe^{2+}. The mixture was incubated for 10 min. The absorbance of the red ferrozine-Fe^{2+}complex was determined using a UV spectrophotometer at 562 nm[45]. Ethylenediaminetetraacetic acid (EDTA) was employed as positive control and deionised water as blank. A standard curve was built using different concentrations of EDTA solution in the range of 0 to 855 μM. Regression of the standard curve gave a linear equation (with $R^2 = 0.9726$) represented by:

$$Y = -0.002X+1.7779$$

The chelating activities of the polysaccharide samples were determined as the EDTA equivalence (μM) using the above equation.

Immunomodulatory activity assays: Procedure for the preparation and maintenance of mouse macrophages (RAW 264.7) was similar to that published in the literature[17,19,45].

Production of IL-6: ELISA kit (IL-6, BD Biosciences, San Jose, CA, USA) was used to measure the concentration of IL-6 as per

the procedure provided in the manufacturer's manual. All measurements were conducted in triplicate[19,45]. Standard IL-6 (mouse) was used to produce the calibration curve that gave a linear equation (with $R^2 = 0.9887$) represented by:

$$Y = 0.0018X+0.0294$$

The concentrations of IL-6 produced by the polysaccharide extracts were calculated using the above equation. The EC_{50} values were then computed from dose dependant production of IL-6.

Production of TNF-α: ELISA kit (TNF-α, BD Biosciences, San Jose, CA, USA) was used to measure the concentration of TNF-α as per the procedure provided in the manufacturer's manual[17,45]. All measurements were conducted in triplicate.

Standard TNF-α (mouse) was used to produce the calibration curve that gave a linear equation (with $R^2 = 0.9879$) represented by:

$$Y = 0.0015Y+0.0734$$

The concentration of TNF-α produced by the polysaccharide extracts was calculated using the above equation. The EC_{50} values were then computed from dose dependant production of TNF-α.

Toxicity test: The viability of macrophage cells (RAW 264.7) against the polysaccharide samples were measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay[17,46]. The absorbance of the samples was measured at 595 nm using the following equation:

$$\text{Cell viability (\%)} = \frac{\text{OD of sample}}{\text{OD of pos control}} \times 100\%$$

The positive control was mouse macrophages treated by only the DMEM medium (without LPS and sample).

***In vitro* anticancer assays against various cancer cell lines:** The cancer cell lines were cultured and incubated according to procedure outlined in a previous publication[4,17]. All the cancer cell lines studied in this research were purchased from the American Type Culture Collection (ATCC, Manassas, VA, USA). Detailed methodology used for these assays were similar to that published previously[4,17].

Optical density of the cells treated with polysaccharide samples was determined at 570 nm using a spectrofluorometric method. The percentage inhibition against various cancer cells was calculated using the following equation:

$$\text{Inhibition (\%)} = \frac{OD_{sample} - OD_{pos\ contr}}{OD_{Neg\ contr} - OD_{pos\ contr}} \times 100$$

Where, $OD_{NegContr}$ is the optical density of the negative control and $OD_{PosContr}$ is the optical density of the positive control. The culture medium containing DMSO (1%) was used as negative control and the medium with 2 mM MMS was used as positive control. IC_{50} values were then computed from dose dependant percentage inhibition.

Statistical analysis: All measurements were made in triplicate and the Mean±SD were determined. The group mean was compared using a one-way analysis of variance (ANOVA) and Duncan's multiple range tests. Statistical calculations were performed using IBM SPSS, OriginPro 8.5 (OriginLab Corporation, Northampton, USA) and Excel 2016 (Official Microsoft, USA). The data were considered to be statistically significant if $p<0.05$.

RESULTS AND DISCUSSION

Chemical composition and monosaccharide content of polysaccharides: The total sugar content of polysaccharides extracted from the selected herbs was measured using the phenol-sulfuric acid method[39] and the results were given in Table 2. It is evident that most polysaccharides contained significant quantities of total sugar (>50%) except *R. rubescens*. The monosaccharide composition of the polysaccharides (monosaccharide standards used and were rhamnose, ribose, fucose, arabinose, xylose, mannose, glucose and galactose) was shown in Table 2. It was interesting to note that glucose is the chief constituent in most of the polysaccharides studied. Significant amounts of galactose, mannose and arabinose were also present in most polysaccharides. Ribose is absent in all the polysaccharides.

Antioxidant activities of herbal polysaccharides

Scavenging abilities against $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radicals: $DPPH^{\bullet}$ and $ABTS^{\bullet+}$ radical scavenging abilities of the polysaccharides were provided in Table 3. It was clear that the majority of the polysaccharides examined have effectively scavenged $DPPH^{\bullet}$ radicals and these activities ranged from 109-178 μM ascorbate equivalent. Their $ABTS^{\bullet+}$ scavenging activities ranged from 158-296 μM ascorbate equivalent.

Table 2: Chemical composition and monosaccharide content of crude polysaccharides extract from dried plant material

Plant numbers	Total carbohydrate content (%)*	Rhamnose (%)	Ribose (%)	Fucose (%)	Arabinose (%)	Xylose (%)	Mannose (%)	Glucose (%)	Galactose (%)
P1	85.65±5.83				1.21		0.89	97.36	0.54
P2	89.58±4.38				1.29	1.25	0.74	95.84	0.88
P3	53.32±2.10	5.32			24.24	4.24	9.88	35.79	20.53
P4	79.07±0.94	13.00			16.75	13.08	12.10	19.05	26.02
P5	53.36±1.11	6.68			14.64	6.00	7.62	47.05	18.01
P6	77.32±4.90	2.23			9.82		3.50	77.46	6.99
P7	98.72±3.34				1.26			98.74	
P8	82.51±1.62	2.62			7.40	1.35	2.83	76.96	8.84
P9	95.39±7.08	3.80			18.03	2.29		62.34	13.54
P10	94.38±1.06	3.54			10.01	1.45	11.04	65.09	8.84
P11	75.48±0.91				4.72		2.06	86.33	6.89
P12	38.62±6.33	4.34			19.29	3.27	2.14	60.02	10.94
P13	91.85±3.59	2.64			12.02			75.62	9.72
P14	65.75±1.36	9.12		24.10				40.03	26.75
P15	78.99±3.99	8.89		36.00		10.63		24.91	19.57
P16	83.32±4.37			2.96	18.03	2.68	5.73	45.93	24.67

*These polysaccharides have combined protein. The numbers represent percentage carbohydrate content and the remaining portion is protein content

Table 3: Antioxidant activities of crude polysaccharides extracted from several medicinal plants

Plant number	#DPPH scavenging activity (Ascorbate equivalent (μM))	#ABTS scavenging activity (Ascorbate equivalent (μM))	&Chelating activity (EDTA equivalent (μM))
P1	124.06±1.88	258.38±0.25	395.45±0.01
P2	146.77±1.57	231.86±0.66	306.95±0.02
P3	154.48±2.53	274.61±0.43	606.28±1.53
P4	167.40±1.44	296.78±0.87	597.28±0.29
P5	154.27±0.72	256.59±0.18	562.62±0.76
P6	147.60±2.19	261.57±0.43	306.95±0.02
P7	13.85±1.30	41.28±1.53	306.95±0.03
P8	110.73±1.05	210.26±1.15	405.95±0.50
P9	66.35±0.95	80.70±0.43	306.95±0.01
P10	109.90±1.57	158.96±1.15	602.28±0.29
P11	158.85±1.30	276.35±1.57	601.78±1.26
P12	178.44±1.88	287.07±0.66	446.28±1.61
P13	133.85±2.82	237.80±1.00	414.12±0.29
P14	161.35±2.01	282.87±0.43	391.95±0.50
P15	109.69±1.88	197.94±0.66	612.78±0.29
P16	109.90±3.15	214.46±0.66	615.62±0.76

#DPPH, ABTS free radical scavenging activity was expressed as equivalent of ascorbic acid, &chelating activity was measured with equivalent of EDTA, Values are: Mean±standard deviation (n = 3)

Highly effective radical scavenging abilities were displayed by polysaccharides isolated from *A. annua*, *A. scoparia*, *A. vulgaris*, *R. rubescens*, *P. cuspidatum*, *S. suberectus* and *A. rugosum* (Table 3). However, polysaccharides extracted from *C. aromatica* and *C. rotundus* showed low scavenging activities.

Interestingly, the results presented in Table 2 and 3 indicated that the most active polysaccharides contain large quantities of glucose and galactose and average quantities of mannose. This observation suggested that the presence of these monosaccharides may be responsible for the observed radical scavenging activities[47,48]. Numerous scientific studies demonstrate that galactose, glucose and mannose do indeed contribute to the radical scavenging abilities of plant based polysaccharides[15,46,49,50]. Chen *et al.*[48] demonstrated that polysaccharides isolated from *Elaegnus angustifolia* L. displayed significant radical scavenging activities and contained a large quantity of galactose, glucose and mannose. Thambiraj *et al.*[46] showed that the polysaccharides extracted from *Lupinus angustifolius* displayed high antioxidant activities and contained large quantities of galactose and significant quantities of mannose and glucose. However, it should be noted that the actual structures of polysaccharides (such as the type of glycosidic linkage and branching) were the main factors that determine the activities of herbal polysaccharides[15,16].

Fe^{2+}chelating assay: The results of the chelating activity of polysaccharides are presented in Table 3. The Fe^{2+} chelating ability of the polysaccharide extracts were determined by

Table 4: Immunomodulatory activities of polysaccharides extracted from selected medicinal herbs**

Plant numbers	EC_{50} for the IL-6 production (μg mL^{-1})*	Cell viability (% of cell survival)#	EC_{50} for TNF-α production (μg mL^{-1})*	Cell viability (% of cell survival)#
P1	266.59±4.00	95.17±3.55	324.60±3.37	97.97±0.50
P2	322.78±4.78	94.80±6.82	334.94±4.59	95.17±1.53
P3	107.78±4.94	90.90±6.20	107.35±5.26	74.97±2.52
P4	337.82±1.40	96.07±7.83	347.75±3.10	94.67±3.51
P5	159.95±2.00	97.77±1.17	203.82±4.50	86.67±4.49
P6	94.23±3.40	93.87±5.04	79.56±4.52	95.07±4.24
P7	337.37±1.46	86.50±5.63	308.14±4.52	68.50±0.50
P8	160.37±4.52	98.53±6.69	104.18±5.48	76.20±2.55
P9	113.52±5.31	94.87±4.76	193.09±2.29	65.73±2.97
P10	93.23±4.22	93.70±3.94	61.74±1.19	87.33±2.08
P11	62.17±2.99	93.23±10.51	174.91±2.89	76.53±5.71
P12	233.79±5.22	93.50±7.13	130.93±3.90	78.13±4.92
P13	175.13±5.05	88.43±0.45	118.24±5.56	96.5±5.070
P14	59.28±0.51	98.00±14.29	62.36±2.30	75.3±5.980
P15	113.52±5.31	91.50±6.00	263.13±2.02	96.4±4.610
P16	62.50±2.43	94.87±1.79	62.09±4.27	96.43±1.40

*IL-6 and TNF-α production was expressed in terms of EC_{50} values, **All measurements were performed in triplicate (n = 3), #Cell viabilities are measured at the 1mg mL^{-1} concentration of herbal polysaccharides

the spectrophotometric method. Among the polysaccharides studied, *A. annua*, *A. scoparia*, *A. vulgaris*, *L. chinensis*, *P. cuspidatum*, *X. sibiricum* and *A. rugosum* showed significant Fe^{2+} chelating capacity. It is clear from Table 2 and 3 that galactose and glucose are the most likely candidates for the chelating abilities of these polysaccharides[46].

Literature reports indicated that the antioxidant capacities of natural products were determined by their combined abilities to scavenge radicals and to chelate with iron[46,51]. Most of the herbal polysaccharides studied in this research displayed significant radical scavenging abilities as well as Fe^{2+}chelating potential. Hence, it is expected that the herbal polysaccharides considered in this study would possess high antioxidant capacities.

Immunomodulatory activities of crude polysaccharides

Effect of herbal polysaccharides to activate mouse macrophages and produce TNF-α and IL-6: Strong evidence exists in the literature to indicate that botanical polysaccharides can activate the immune system to produce various cytokines[5,15,31].Treatment of RAW 264.7 cells with polysaccharides from the 16 medicinal herbs showed concentration-dependent enhancement of the production of TNF-α and IL-6 (Table 4 and Fig. 2).

The immunomodulatory activities were measured at different concentrations (0-1 mg mL^{-1}) and expressed in term of EC_{50} values (Table 4). Toxicities of various polysaccharides are also given in Table 4 and the results indicate that most of the isolated polysaccharides from 16 herbs display significant immunostimulatory activity by increasing the production of TNF-α and IL-6. Polysaccharides from *A. annua*, *A. rugosum*, *L. chinensis*, *C. reticulata* and *S. suberectus* showed high immunostimulatory activities as evidenced by the production of TNF-α and IL-6 (EC_{50} values less than 110 μg mL^{-1}). Polysaccharides from the remaining herbs also displayed significant immunostimulatory activities (Table 4).

The concentration dependant immunostimulatory activities of the most active herbs are shown in Fig. 2. Polysaccharides from *L. chinensis* and *S. suberectus* display very high activity as indicated by their TNF-α production. The polysaccharides of *A. rugosum* and *A. annua* also led to the production of highly significant quantities of TNF-α. With respect to the production of IL-6, the polysaccharides from *A. rugosum* and *A. annua* were the best.

It was therefore expected that the herbal polysaccharides from *A. rugosum*, *A. annua*, *L. chinensis* and *S. suberectus* have high potential to be used as immunostimulators and hence were potential candidates for cancer therapy[5,15,16]. These observations are strongly supported by the literature, for instance, lentinan is one of the well-known immuno-enhancing mushroom polysaccharide that has been successfully used in chemo-immunotherapy in combination with fluoropyrimidine to improve survival rates of patients with gastric cancer[21]. It is therefore expected that the polysaccharides from *A. rugosum*, *A. annua*, *L. chinensis* and *S. suberectus* were excellent candidates for the formulation of immuno-chemotherapeutic agents.

Toxicities of polysaccharides: The toxicities of the extracted polysaccharides are given in Table 4. Cell viabilities were measured at 1 mg mL^{-1}concentration of polysaccharide. The

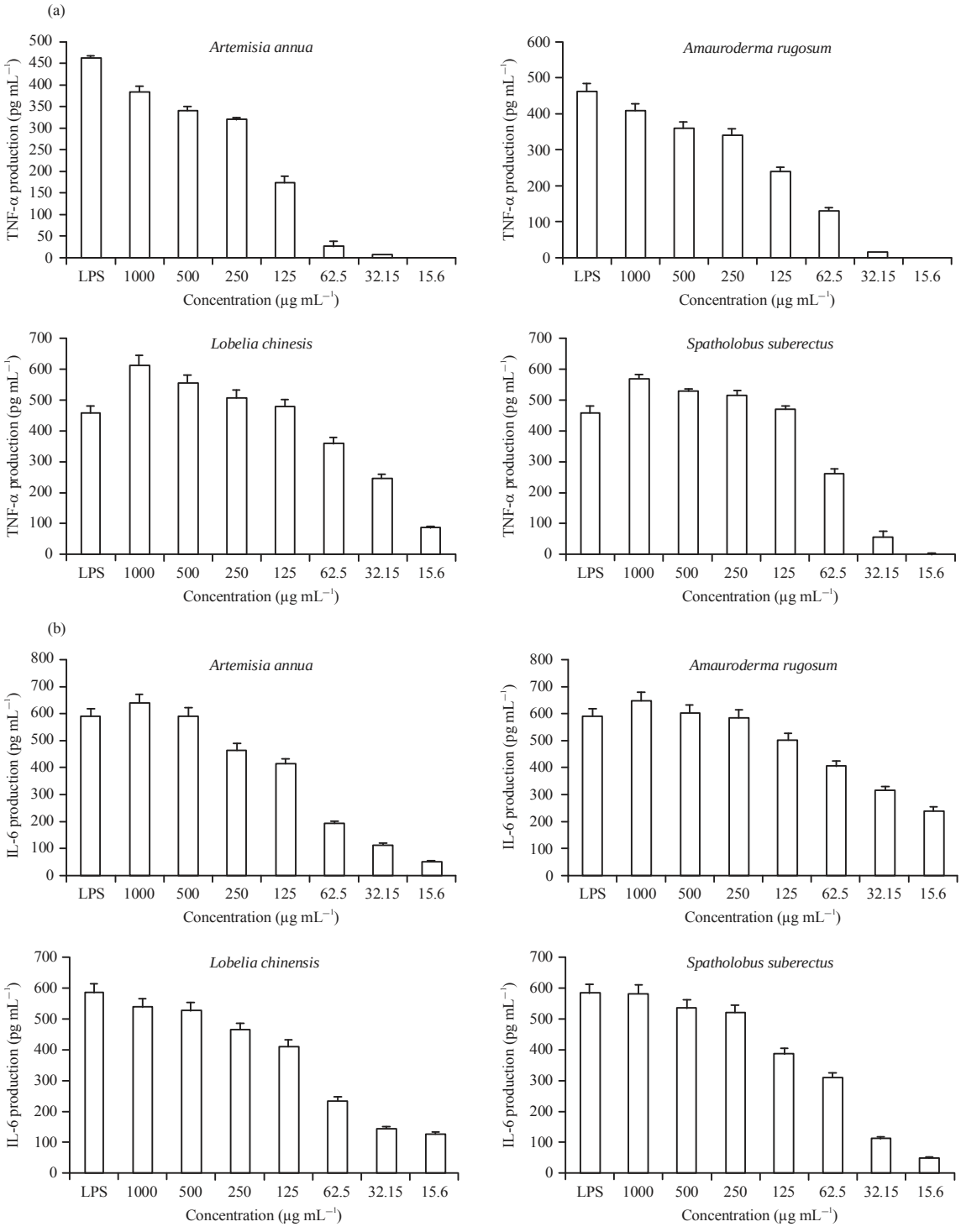

Fig. 2(a-b): Concentration dependant immunomodulatory activities of most active polysaccharide extracts, (a) TNF-α production and (b) IL-6 production. Results are given as Mean±SD (n = 3), p<0.05 is considered to be statistically significant

results demonstrated that the herbal polysaccharides studied in this study displayed good cell viabilities (Table 4) indicating low toxicities. These results are in agreement with literature findings[5,13-16].

Anticancer activities of herbal polysaccharides: The anticancer activities of the polysaccharides isolated from the medicinal herbs were measured against five cancer cell lines, namely, A549 (lung carcinoma), MCF7 (breast

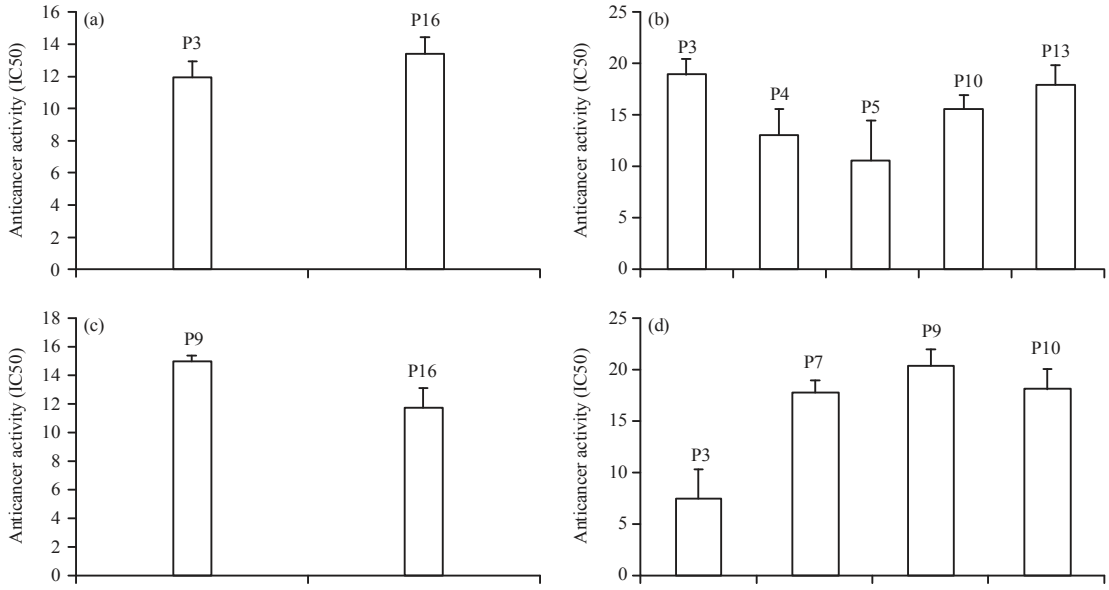

Fig. 3(a-d): Anticancer activities (IC_{50}) of polysaccharide extracts against four different cancer cell lines, (a) A549, (b) MCF7, (c) HT29 and (d) MiaPAca2

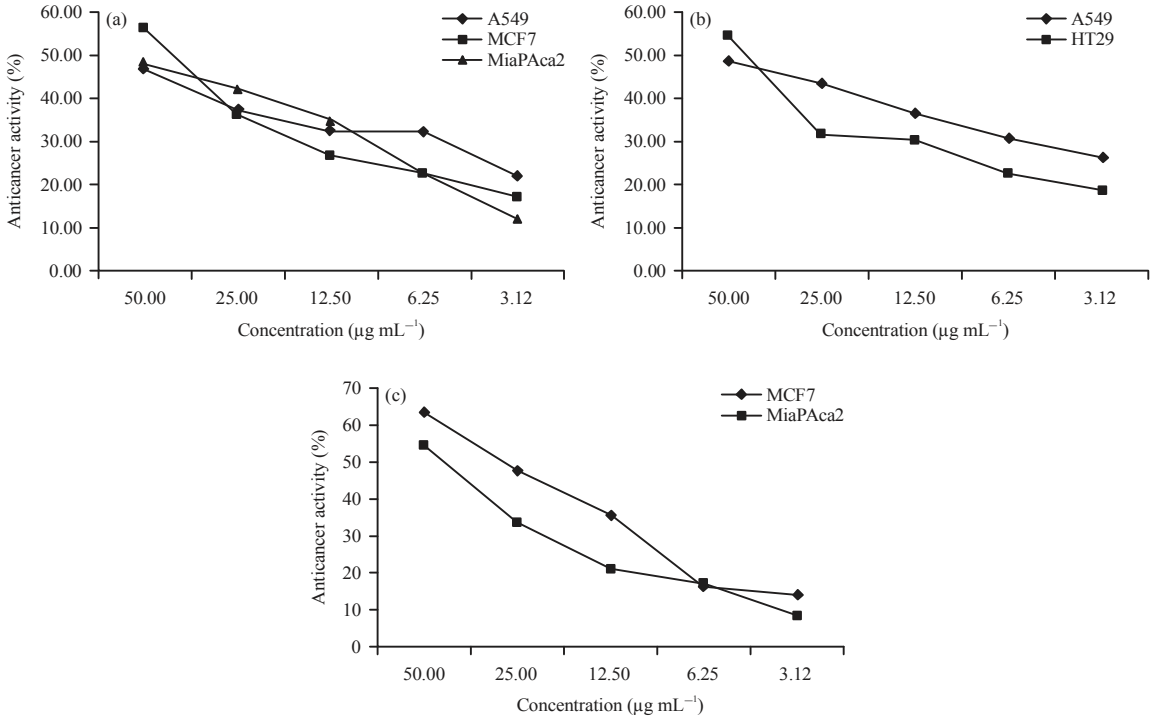

Fig. 4(a-c): Dose dependant variation of anticancer activities of polysaccharides from the three most active herbs. Anticancer activity of polysaccharides from, (a) *A. annua*, (b) *A. rugosum* and (c) *L. chinensis*

carcinoma), HT29 (colon carcinoma), HepG2 (hepatocytes carcinoma) and MiaPAca2 (pancreatic carcinoma) and the results are presented in Fig. 3 and 4.

The polysaccharides from *A. annua*, *A. rugosum*, *C. rotundus* and *L. chinensis* displayed significant anticancer activities against two or more cancer cell lines (Fig. 3). It is interesting to note from Fig. 3 and 4 that the polysaccharides extracted from *A. annua* displayed very high anticancer activity against A529 (lung carcinoma) and MiaPAca2 (pancreatic carcinoma).

The biological activities of polysaccharides are dependent on their structure[15,16]. The structures of polysaccharides are in turn related to the type and quantities of their monosaccharide constituents[16]. It is therefore expected that the monosaccharide content may be indirectly related to the activities of the polysaccharides. An examination of the anticancer activities (Fig. 3) and the monosaccharide content (Table 2) indicate that galactose, glucose, mannose and arabinose are likely to contribute towards anticancer activity. For example, the extracts of *A. annua*, *A. scoparia*, *A. vulgaris* and *L. chinensis* which show good anticancer activity also contain significant quantities of these four monosaccharides (galactose, glucose, mannose and arabinose) (Table 2 and Fig. 3). The extracts of *C. rotundus*, *R. palmatum* and *A. rugosum* showed significant anticancer activity and these also contain significant quantities of at least three of these monosaccharides (galactose, glucose and arabinose/mannose) (Table 2 and Fig. 3). Similar correlations were observed for immunostimulatory and antioxidant activities.

It was important to examine the findings of this study in light of the published literature[10,15,20,21,22]. Three of the herbal polysaccharides studied here (namely, *A. annua*, *L. chinesis* and *A. rugosum*) exhibited significant immunostimulatory as well as anticancer activities. Hence, they were potential candidates for the discovery of immunostimulatory polysaccharides for cancer therapy.

CONCLUSION

In this study, polysaccharides from selected TCM herbs have been investigated for their antioxidant, immunomodulatory and anticancer activities. Polysaccharides from *A. annua*, *A. rugosum*, *L. chinensis*, *S. suberectus*, *A. scoparia*, *P. cuspidatum* and *X. sibiricum* showed significant radical scavenging and Fe^{2+}chelating activities indicating that these polysaccharide extracts have significant antioxidant potential. In addition, the immunomodulatory activities revealed that the polysaccharides from *A. annua*, *A. rugosum, L. chinensis*, *C. reticulata* and *S. suberectus* exhibit significant stimulation of mouse macrophages to produce TNF-α and IL-6.It was interesting to note that monosaccharides such as galactose, glucose, mannose and arabinose are most likely to be responsible for the antioxidant and immunomodulatory activities. The polysaccharides extracted from *A. annua*, *A. rugosum* and *L. chinensis* showed significant anticancer activities against two or more cancer cell lines. The polysaccharides from these herbs also showed significant antioxidant and immunomodulatory activities. Hence, these three herbs have great potential for the isolation of anticancer polysaccharides with immuno-enhancing capabilities.

SIGNIFICANCE STATEMENT

Sixteen TCM herbs were carefully selected for this study based on the available traditional knowledge on their anticancer and immunomodulatory properties. Polysaccharides from a majority of these herbs are yet to be systematically examined using contemporary scientific tools. TCM practitioners commonly use hot water extracts for the treatment of cancer and other diseases. Abundant traditional knowledge on the selected herbs and the limited scientific understanding of their polysaccharides warrant further study. Therefore, this research aimed to extract water-soluble polysaccharides and examine their immunostimulatory and anticancer properties. Major significance of this research is to determine the best herbs that contain novel polysaccharides with immune-enhancing and anticancer properties that will ultimately lead to the discovery of immuno-chemotherapeutic agents. Cancer therapy is expensive and the patients from many developing countries cannot afford the cost. Hence, the discovery of novel therapeutics from the medicinal herbs will provide great benefit to the humanity.

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