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Research Article
Antioxidant and Immunomodulatory Activities and Structural Characterization of Polysaccharides Isolated from *Lobelia chinensis* Lour

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Abstract

Background and Objective: *Lobelia chinensis* Lour is an important anticancer herb used in traditional Chinese medicine. Many botanical polysaccharides are known to exhibit immunomodulatory and anticancer activities. This research aimed to analyze the *L. chinensis* polysaccharides (LCPs) for their biological activities relevant to their anticancer function. **Materials and Methods:** Water-soluble LCPs were extracted and purified using size-exclusion chromatography to obtain two dominant polysaccharides, LCP-1 and LCP-2 having molecular masses of 1899 kDa and 5.3 kDa, respectively. The antioxidant potentials of the isolated polysaccharides were evaluated by measuring radical scavenging activities against DPPH$^{\bullet}$ (2,2-diphenyl-1-picrylhydrazyl radical), ABTS$^{\bullet+}$(2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid radical) and OH$^{\bullet}$ (hydroxyl radical). Immunostimulatory activities of LCP-1 and LCP-2 were measured using mouse macrophages. Structure of the most active fraction (LCP-2) was determined using FT-IR and NMR spectroscopic techniques. **Results:** Two isolated polysaccharide fractions displayed significant antioxidant activities and stimulated the production of tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6), although LCP-2 is more effective. Detailed structural characterization by FT-IR and NMR was undertaken for the most active fraction (LCP-2) and confirmed that LCP-2 is(2,1)-β-fructan. **Conclusion:** The results suggested that the polysaccharides isolated from *Lobelia chinensis* Lour are potential candidates for immune-chemotherapy and suitable for the treatment of cancer.

Key words: *Lobelia chinensis* Lour, polysaccharides, immunostimulatory effects, antioxidant activities, FT-IR spectroscopy, NMR spectroscopy

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Competing Interest: The authors have declared that no competing interest exists.

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

INTRODUCTION

Lobelia chinensis Lour (Campanulaceae) is an important anticancer herb which has been widely used as diuretic, hemostat, antidote and anticancer agent in Traditional Chinese Medicine (TCM) for decades[1,2]. This herb is used in several traditional anticancer formulations to treat gastric cancer, lung cancer, colorectal cancer and liver cancer[1,3]. Modern scientific studies revealed that *L. chinensis* contains several important classes of bioactive compounds such as piperidine alkaloids, flavonoids, terpenoids and coumarins[4-6]. Several studies demonstrated that the extracts from this herb displayed a number of pharmacological activities such as antibacterial, anti-venom and anticancer activities[4-6]. The hot water extracts (decoction) from *L. chinensis* have been shown to display significant immunostimulatory and anticancer properties against liver cancer (H22) and Gastric cancer (BC-38)[4,7,8]. Preliminary scientific studies from authors' laboratory indicated that the water extracts from *L. chinensis* have shown high antioxidant and immunostimulatory activities[9-11]. However, the literature on the polysaccharides from *L. chinensis* and their characterization is very limited[1].

Literature demonstrates that botanical polysaccharides exhibit a variety of pharmacological activities that include anticancer, immune regulation and antioxidant activities[12-21]. In recent decades, herbal polysaccharides are proving to be ideal candidates for anticancer agents due to their relevant biological activities with minimal side effects[1,14,21-26]. Many polysaccharides such as lentinan, Polysaccharide Krestin (PSK), Polysaccharopeptide (PSP) and schizophyllan, isolated from medicinal mushrooms are in clinical use as anticancer agents due to their excellent anticancer properties along with immuno-regulatory effects[12-14,21,27]. Therefore, it is of enormous interest to study polysaccharides from traditional herbs that are promising candidates to develop novel therapeutics for the treatment of cancer as well as immune-regulation. To the best of our knowledge there is only one publication involving isolation of an α-Glucan from *L. chinensis* that displayed significant immunostimulatory activity[1].

It is known in the literature that the plant extracts which show antioxidant and immunomodulatory potential, concurrently exhibit anticancer effects[14,17,19-21,28-31]. Therefore, the screening for potential immunomodulators is one of the important steps for the development of anticancer therapeutics. Hence, the objectives of this study were to isolate polysaccharides from *L. chinensis* and evaluate their antioxidant and immunostimulatory activities. It is also aimed to carry out structural characterization of *L. chinensis* polysaccharides using FT-IR and NMR spectroscopic techniques with a view to understand structure-activity relationship.

MATERIALS AND METHODS

This research was carried out between March 2015 to November 2017 as part of the program for the discovery of novel anticancer agents in Authors' Laboratory.

Procurement of medicinal plant associated with this research: *Lobelia chinensis* Lour was purchased from Bei Jing Tong Ren Tang, a Chinese Herbal Medical Centre located in Sydney (Australia). This company has branches all over the world and is well known for their best practice in TCM. The herbs traded in Sydney centre have approvals from both Australian and Chinese Governments. The company undertakes stringent authentication and quality control procedures for all the herbal materials supplied by them.

Chemicals and materials: The 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), dimethyl sulfoxide (DMSO), 1,10-phenanthroline, H_2O_2, ferrozine, 95% ethanol, ascorbic acid, sulfanilamide, N-(1-1-napthyl) ethylenediamine dihydrochloride, lipopolysaccharide (LPS) were purchased from Sigma (Australia) and Lomb Scientific Pvt., 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.

Extraction and fractionation of polysaccharides from *L. chinensis*: To extract water-soluble compounds, 25 g of *L. chinensis* Lour was powdered then autoclaved (121°C, 2 h). The extract was cooled to laboratory temperature and the supernatant was separated by filtration and then crude compounds were precipitated by treating the supernatant with 95% ethanol (Fig. 1). The isolated polysaccharides were de-proteinated using Sevag method[21] and purified using gel filtration (Sepharose CL-6B; 2.4×99 cm, flow rate: 0.51 mL min^{-1}). Details of the procedure followed for the extraction and purification of polysaccharides is similar to that published previously[16,17,19-21] and described in Fig. 1. Polysaccharide fractions were obtained based on the sugar profile of the eluted samples[19-21]. These fractions were collected, freeze-dried and then kept at -20°C until further studies.

Determination of average molecular mass: Molecular weights of these fractions were determined by calibrating the

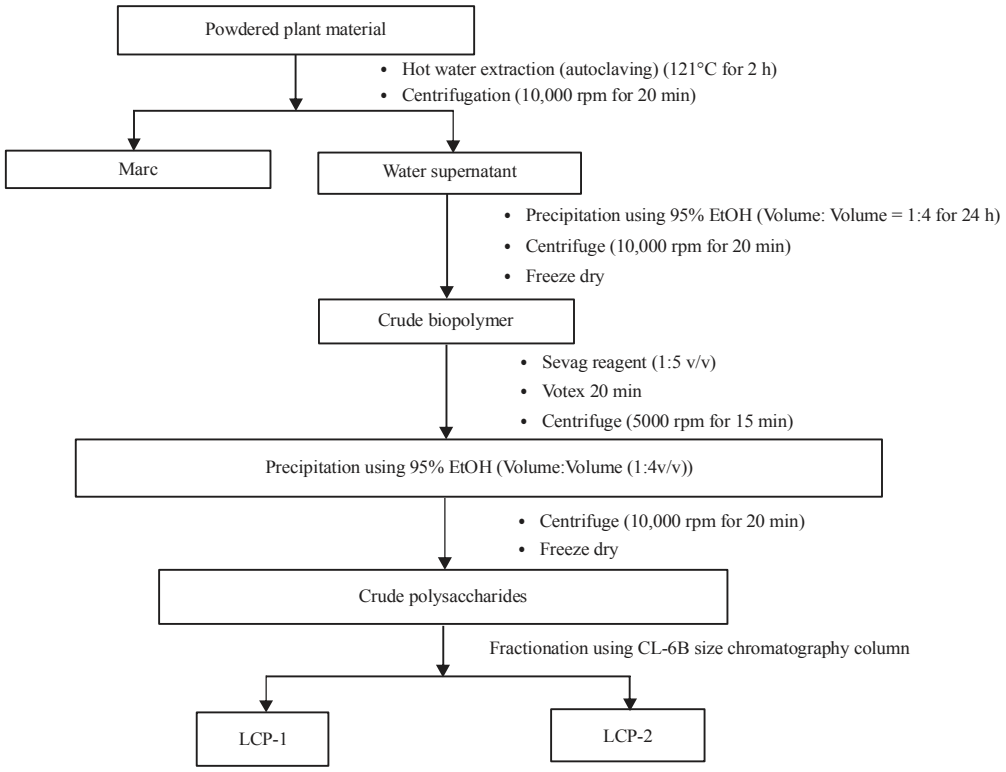

Fig. 1: Flow chart for the extraction of polysaccharides from *L. chinensis*

Sepharose CL-6B gel filtration column. Standard dextrans with average molecular weight (from 2000 to 1 kDa) were used to obtain a calibration curve[16,17,19-21]. Regression of the standard curve gave a linear equation (with $R^2 = 0.9915$) represented by:

$$y = -0.2763x+1.8022$$

This equation was used to estimate the average molecular weight of polysaccharides obtained from *L. chinensis* (LCPs).

Determination of chemical composition: The total sugar content was measured using phenol-sulfuric acid method[16,17,19-21,32]. Glucose was used to produce a standard curve that was used to determine the sugar contents. Regression of the standard curve gave a linear equation (with $R^2 = 0.9964$) represented by:

$$y = 0.0018x+0.0374$$

The total protein contents were measured using modified Lowry's method, using BSA to prepare standards[17,19-21,33]. BSA was used to build a standard equation that was used to determine the bound protein. Regression of the standard curve gave a linear equation (with $R^2 = 0.9923$) represented by:

$$y = 0.0017x-0.0212$$

The mono-sugar contents were determined by gas chromatography (Hewlett Packard 7890B) with FID detection[16]. The approach followed to prepare the samples for GC analysis was based on the procedure published previously[16,21,34]. Mannose, glucose, galactose, xylose, fucose, rhamnose, arabinose and ribose were used as mono-sugar standards.

FT-IR analysis: A TENSOR II FTIR Spectrometer (BRUKER) was used for structural characterization of LCP's at room temperature (25°C)[16]. All spectra were recorded between the frequency range of 4000-450 cm^{-1}.

NMR analysis: ^{1}H, ^{13}C, g-COSY and HSQC spectra were recorded using Bruker Avance 400 MHz NMR spectrometer using an inverse detection probe with pulsed field gradient capabilities. LCP-2 (25 mg) was dissolved in 600 µL of D_2O (99.9%) containing 0.15% TSP (v/v ratio) and all NMR experiments were performed at 40°C.

Bioactivity tests

DPPH• scavenging assay: The Blois method[35] was used to determine the DPPH• scavenging abilities of polysaccharides. The procedure employed for this assay was similar to previously published methods[17,19,20,36]. 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 LCPs was determined as the ascorbic acid equivalence using the above equation.

ABTS•+ radical scavenging assay: ABTS•+ scavenging abilities of polysaccharides were determined using published[10,11,36,37]. 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 734nm[10,11] (Multiskan 141 EX, Thermo Electron, USA).

Regression of the data gave a linear standard curve (with R^2 = 0.9699) represented by the following equation:

$$y = -0.0017x+0.716$$

The ABTS•+ scavenging capacities of LCPs were determined as the ascorbic acid equivalence using the above equation.

OH• radical scavenging test: The OH• scavenging capacity of LCPs was measured employing the method described by De Avellar *et al.*[38] and Pownall *et al.*[39] with minor modification. Briefly, 50 μL of polysaccharide fractions or ascorbic acid (1 mg mL^{-1}) were added with 50 μL of 3 mM 1,10-phenanthroline and 50 μL of 3 mM $FeSO_4$ in a 96 well microtiter plate. About 50 μL of H_2O_2 (0.01% v/v) was then added and mixed to trigger the competition between polysaccharides[38] and Fe^{2+} for OH•. The solution was incubated at ~37°C for 60 min. The absorbance values were measured at 536 nm using UV-spectrophotometer. The OH• scavenging ability of LCPs was determined employing the equation:

$$\text{OH}^{\bullet}\text{ scavenging activity (\%)} = \frac{\text{OD of sample} - \text{OD of neg\_control}}{\text{OD of blank} - \text{OD of neg\_control}} \times 100\%$$

where, the negative control is the reaction mixture without sample and without ascorbic acid. The blank is the reaction mixture without sample, ascorbic acid and H_2O_2.

Immunostimulatory activity assays: Procedure for the preparation and maintenance of mouse macrophages (RAW 264.7) is similar to that published in the literature[10,11,16,40].

IL-6 production: ELISA kit (IL-6, BD Biosciences, San Jose, CA, USA) is then used to measure the concentration of IL-6 as per the procedure provided in the manufacturer's manual[10,11,16,40]. All experiments were conducted in triplicate. Standard IL-6 (mouse) was used to produce the calibration curve that gave a linear equation (with R^2 = 0.992):

$$y = 0.0019x+0.0248$$

The concentrations of IL-6 produced by the polysaccharides were calculated using the above equation.

TNF-α production: ELISA kit (TNF-α, BD Biosciences, San Jose, CA, USA) was then used to measure the concentration of TNF-α as per the method provided in the manual and as previously described[10,11]. Triplicate measurements were conducted.

Standard TNF-α (mouse) was used to produce the calibration curve that gave a linear equation (with R^2 = 0.9875):

$$y = 0.0017x+0.0706$$

The concentration of TNF-α produced by the polysaccharide extracts were calculated using the above equation.

Determination of toxicity by MTT test: Viability of macrophage cells (RAW 264.7) were measured employing the MTT assay as previously described[10,11,16,41]. The absorbance values were the measured at 595 nm and the fraction of live cells was determined 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).

Statistical analysis: All data were measured in triplicate and Mean±SD were determined. A one-way analysis of variance (ANOVA) and Duncan's multiple range tests were used for analysis of data. Statistical calculations were performed using OriginPro 8.5 and Excel 2016. The data were considered to be statistically significant if $p<0.05$.

RESULTS AND DISCUSSION

Extraction and fractionation of polysaccharides from *L. chinensis*: Two polysaccharide fractions, namely, LCP-1 and LCP-2 were isolated from *L. chinensis* using hot water extraction and subsequent purification on a Sepharose CL-6B column (Fig. 2a). As could be seen from the sugar profile (Fig. 2a), LCP-2 is the major polysaccharide fraction. Calibration of the column with dextran standards revealed that the average molecular masses of LCP-1 and LCP-2 were 1899 and 5.3 kDa, respectively (Fig. 2b).

Chemical compositions of the fractions: Total carbohydrate and total protein compositions of isolated fractions (LCP-1 and LCP-2) were analyzed and these results are presented in Table 1. The carbohydrates are the chief constituents of each fraction (94% in LCP-1 and 96% in LCP-2). Table 1 also presented the mono-saccharide compositions of the isolated fractions. It can be seen from these results that LCP-1consisted mainly of Rhamnose (14.39%), Arabinose (24.39%), Galactose

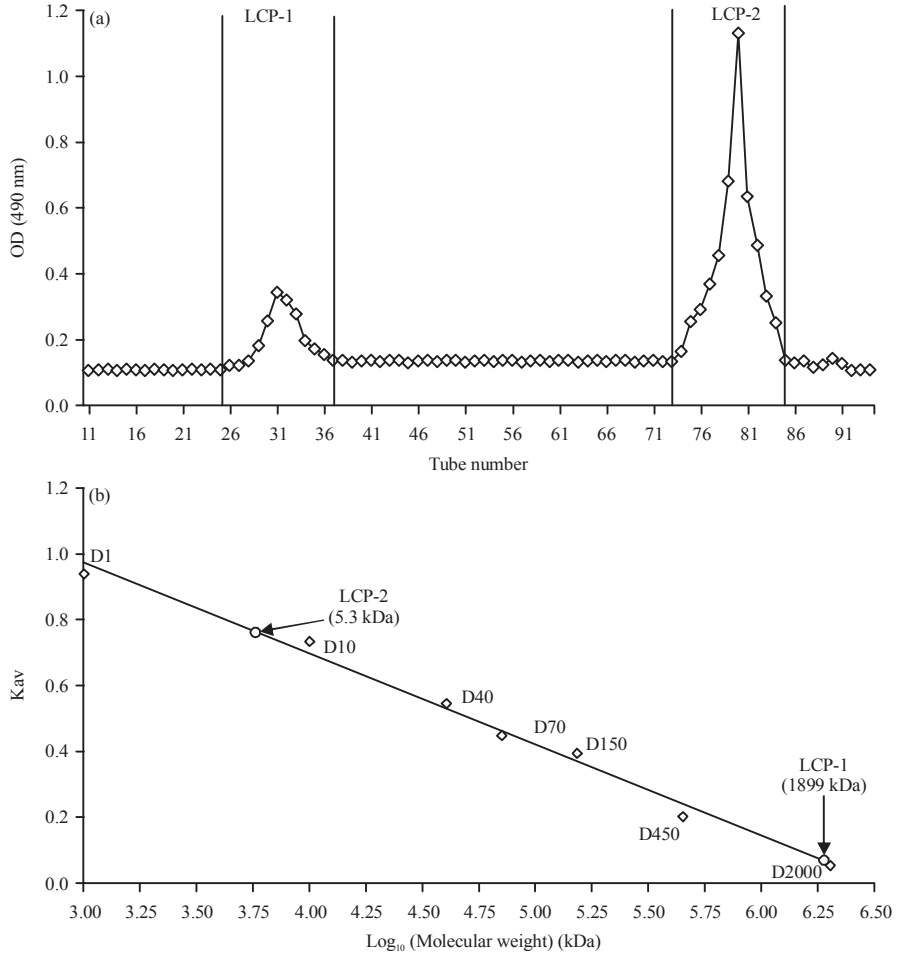

Fig. 2(a-b): (a) Isolation and purification of LCPs using Sepharose CL-6B column (two fractions: LCP-1and LCP-2 were separated) and (b) Standard calibration curve for the calculation of average molecular masses of LCPs based on the elution volume and the molecular mass of standard dextran samples

D2000: 2,000 kDa, D450: 450 kDa, D150: 150 kDa, D70: 70 kDa, D40: 40 kDa, D10: 10 kDa and D1: 1 kDa; Kav: $(V_e-V_o)/(V_t-V_o)$, V_o is void volume of the column, V_t is total column volume, V_e is elution volume of the fraction

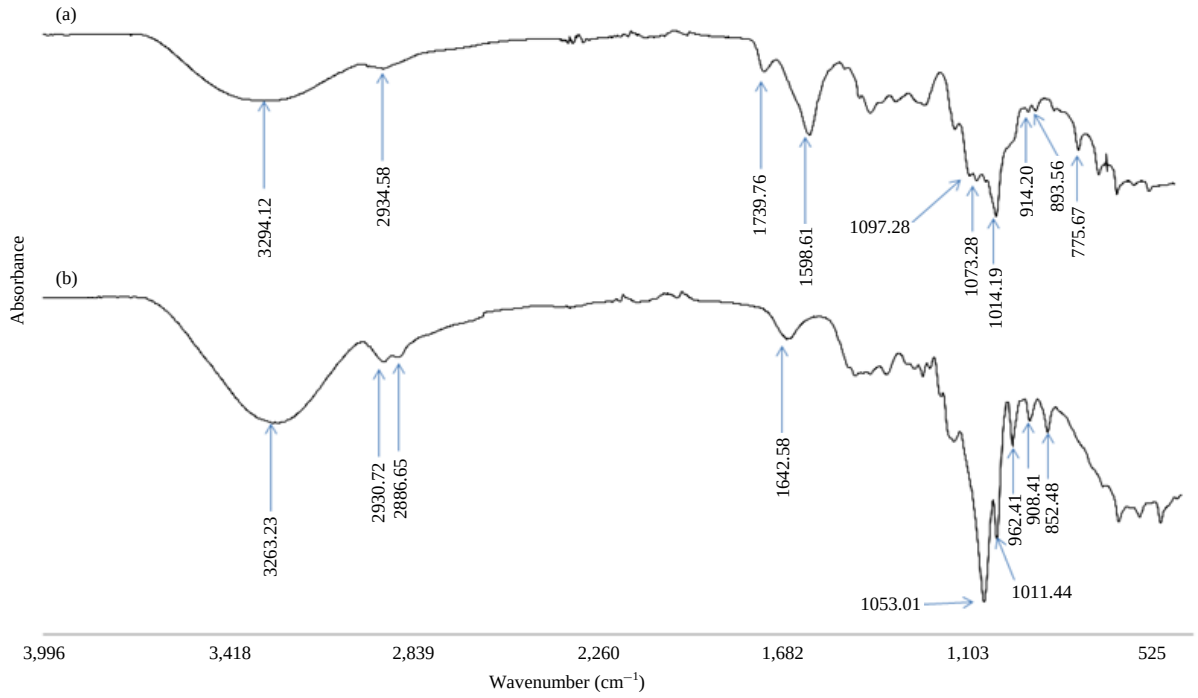

Fig. 3: FTIR spectrum of (a) LCP-1 and (b) LCP-2

Table 1: Chemical composition and monosaccharide contents of LCPs

Parameters (%)	LCP-1	LCP-2*
Total protein	5.95	3.57
Total carbohydrate	94.05	96.43
Monosaccharide (% ratio)		
Rhamnose	14.39	
Fucose		
Ribose		
Arabinose	24.39	
Xylose	1.87	
Mannose	3.31	43.83*
Glucose	18.87	56.17*
Galactose	33.80	
Unknown	3.37	

*Fructose is the major monosaccharide present in LCP-2. It should be noted that, during the reduction step of GC sample preparation, fructose gets reduced to mannitol and glucitol. Therefore, mannose and glucose are seen in GC results instead of fructose

(33.8%) and Glucose (18.87%). However, the main mono-saccharides in LCP-2 were mannose (43.83%) and glucose (56.17%) (Table 1). It is important to note at this point that, reduction of fructose during GC sample preparation yields mannitol and glucitol[42-44]. It is therefore expected that LCP-2 may possibly contain glucomannan and/or fructan. FT-IR and NMR spectroscopic investigations have provided further structural details of LCP-2 indicating the presence of fructose units in this polysaccharide.

Vibrational spectroscopy analysis: Figure 3 provided FT-IR spectra of *L. chinensis* polysaccharides LCP-1 and LCP-2. LCP-1 showed peaks (Fig. 3a) consistent with α-glycosidic linkage (755.6 cm^{-1}) and β-glycosidic linkage (893 and 914 cm^{-1})[16,21,45,46]. The spectrum also showed three strong absorption bands at 1014.19, 1073.28 and 1097.28 cm^{-1} (corresponding to C-Ostretching vibrations related to glycosidic bonds) indicating the presence of a pyranose sugar in LCP-1[16,21,45,46]. The broad peak centred at 3294.12 cm^{-1} corresponds to hydroxyl stretching vibrations of the polysaccharide and the peaks at 2934.58 cm^{-1} are due to C-H stretching vibrations[21,46]. Remaining peaks are consistent with polysaccharide structure. These observations lead to the conclusion that LCP-1 contains pyranose sugars with α- and β-glycosidic linkages. LCP-2 (Fig. 3b) has peaks at 908.41 and 852.48 cm^{-1} indicating the presence of β-glycosidic linkage[16,21,45,46]. The two strong absorption peaks in the range of 1011-1053 cm^{-1} (corresponding to C-O stretching vibrations related to glycosidic bonds) indicate the presence of furanose sugars in LCP-2[21,46]. As can be seen from the vibrational bands in the range 1010-1100 cm^{-1} of the FT-IR spectrum (Fig. 3b), there are no pyranose type sugars[21,46] in LCP-2. The presence of only furanose sugars indicates that LCP-2 might be a fructan[42,43]. The broad band centred on 3263.68 cm^{-1} corresponds to the hydroxyl stretching vibrations of the polysaccharide and the peaks at 2930.72 and 2886.65 cm^{-1} belong to C-H stretching vibrations. These observations confirm that LCP-2 contains furanose sugars with β-glycosidic linkages.

NMR spectroscopy analysis: Results demonstrated that LCP-2 is the most active and major polysaccharide fraction isolated from *L. chinensis*. Therefore, a detailed structural analysis of LCP-2 was carried out by NMR spectroscopy. ^{1}H, ^{13}C, g-COSY and HSQC spectra of LCP-2 were given in Fig. 4. Standard assignment protocols were used to obtain proton and carbon chemical shift assignments (Table 2) using 1D- and 2D-NMR spectra. Absence of intense proton resonances in 4.4-5.5 ppm range (Fig. 4a) indicated that there were no anomeric protons in LCP-2. In addition, LCP-2 showed six intense carbon peaks (Fig. 4b) indicating that it contains a single mono-saccharide unit in the main chain. These observations together with GC and FT-IR results strongly indicate that LCP-2 is a fructan with fructose units in the main chain. The weak anomeric doublet at 5.42 ppm in the proton spectrum (Fig. 4a) is likely due to the presence of a chain terminating glucose residue is the fructan. A set of weak resonances between 3.3-3.6 ppm confirms the terminal glucose residue.

The fructan backbone structure of LCP-2 can be confirmed from proton and carbon connectivities in g-COSY and HSQC spectra. The g-COSY spectrum showed several proton connectivities as shown in Table 3. Clearly, the protons H3 to H6 within the fructofuranosyl ring (Fig. 5) displayed correlations in the g-COSY spectrum. Also, the two allylic protons H1' and H1" attached to C1 showed intense cross peak in the g-COSY spectrum (Table 3, Fig. 4c). Five intense cross peaks are clearly observed for the five protonated carbons in the HSQC spectrum (Table 3, Fig. 4d). The anomeric carbon (C2) at 105.91 ppm (Table 2, Fig. 4b) did not show any cross peak as there are no protons attached to this carbon (Table 3, Fig. 5). Literature showed that β-anomeric ^{13}C resonances are commonly located between[21,46,47] 103 and 105 ppm. Therefore, the large chemical shift value (105.91 ppm) observed for the anomeric carbon demonstrates that LCP-2has a β-glycosidic linkage[21,46,47]. Proton and carbon chemical shifts of LCP-2 (Table 2) are consistent with the correlations observed in 2D-NMR spectra. These assignments together with FT-IR and GC findings lead to following conclusions: (i) GC analysis revealed that LCP-2 may contain glucomannan and/or fructan, (ii) FT-IR results indicated that LCP-2 contains only furanose sugars with β-glycosidic linkage (and no pyranose sugars are present) indicating the presence of fructose units in the backbone with β-linkage and (iii) NMR results together with FT-IR findings confirm that LCP-2 is a β-D-(2→1) fructofuranoside. These results demonstrated that LCP-2 contains the following fructan backbone with glucose in the chain terminating position:

α-D-glucopyranosyl -(1→2)-[β-D-fructofuranosyl-(2, 1)-β-D-fructofuranosyl]$_n$-(2→1)-β-D-fructofuranosyl

The chemical shift values of LCP-2 presented in Table 2 match well with inulin type β-fructans isolated from *Artemisia japonica*[42], *Saussurea costus*[43], *Ophiopogon japonicas*[44] and *Matrisia maritima*[48] confirming the structure proposed for LCP-2 (Fig. 5). This is a significant result reporting isolation of an immunostimulatory fructan for the first time from *Lobelia chinensis*.

Radical scavenging activities: The results of radical scavenging activities of LCPs (against three different radicals) are given in Table 4. It was cleared from the results that, both

Table 2: ^{1}H and ^{13}C chemical shifts (ppm) of LCP-2 from *L. chinensis*

H1'/H1"	C1	H2	C2	H3	C3	H4	C4	H5	C5	H6'/H6"	C6
3.7/3.9	63.66	-	105.91	4.24	79.78	4.08	77.08	3.85	83.78	3.77/3.82	64.83

Table 3: Proton and carbon correlations from 2D-NMR spectra data of LCP-2 from *L. chinensis*

^{1}H-^{1}H connectivities from g-COSY	^{1}H-^{13}C connectivities from HSQC
	C1↔H1
H1'↔H1"	C2↔No cross peak*
H3↔H4	C3↔H3
H4↔H5	C4↔H4
H5↔H6	C5↔H5
	C6↔H6

*There is no proton on C2 (no H2) in fructan

Table 4: Radical scavenging activities of LCPs along with their average molecular mass

Sample (1mg mL^{-1})	Molecular mass (kDa)	ABTS$^{\bullet+}$ scavenging activity (Ascorbic acid equivalent μM)#	DPPH$^{\bullet}$ scavenging activity (Ascorbic acid equivalent μM)#	OH radical scavenging (%)*
LCP-1	1899	206.12±0.29	145.56±2.32	48.21±1.04
LCP-2	5.3	155.78±0.37	103.76±2.31	44.08±0.86

#ABTS and DPPH free radical scavenging activity was expressed as equivalent of ascorbic acid, *The percentage of inhibition of OH production after treatment with polysaccharides, Values: Mean±standard deviation (n = 3)

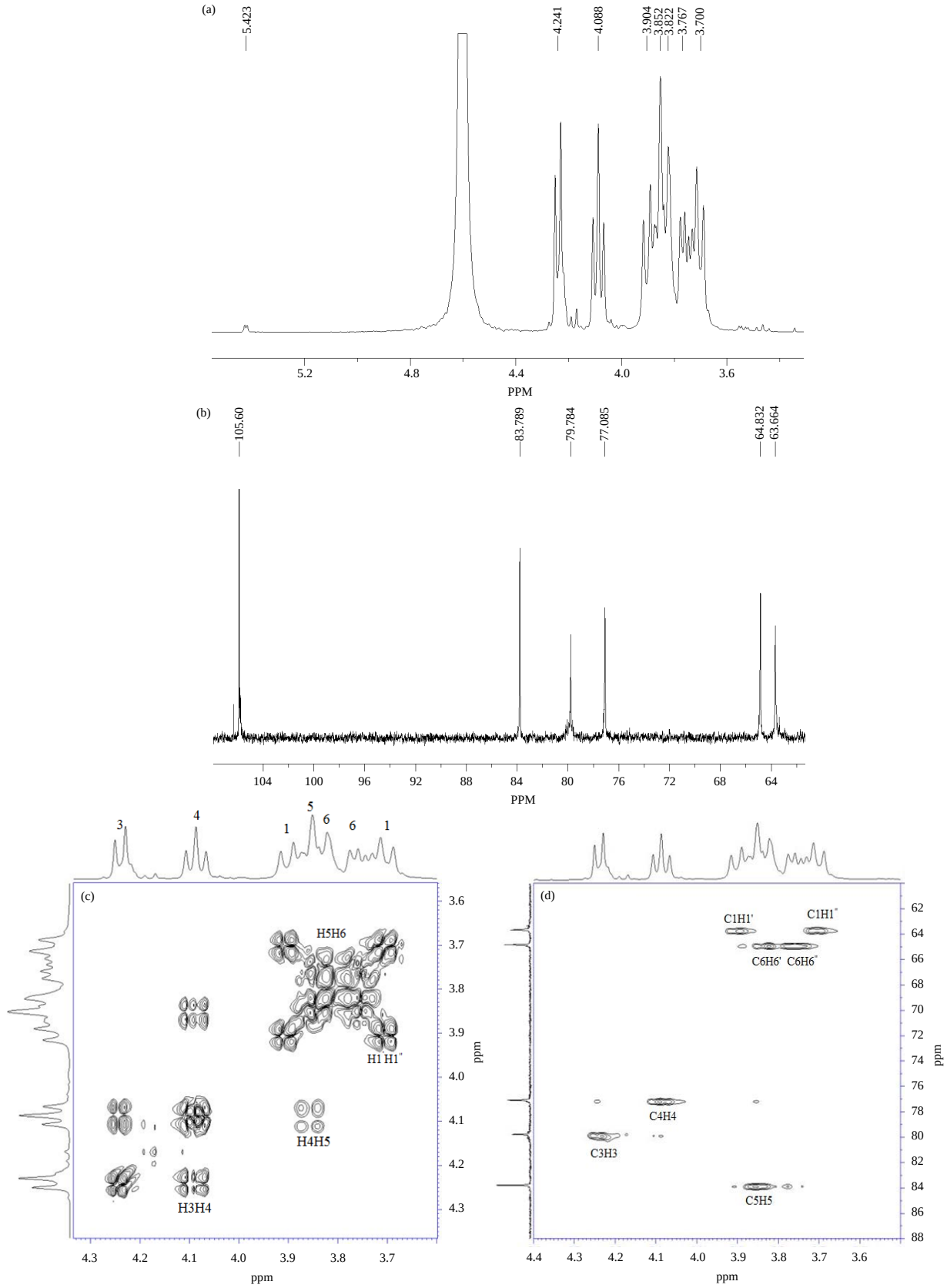

Fig. 4(a-d): NMR spectra of LCP-2 isolated from *L. chinensis* (a) ^{1}H-NMR, (b) ^{13}C-NMR, (c) g-COSY and (d) HSQC

LCP-1 and LCP-2 displayed significant radical scavenging activities against the three radicals tested in this research. LCP-1 exhibited slightly better scavenging activity than LCP-2 (Table 4). An interesting point to be noted here is that the only mono-saccharide present in LCP-2 is fructose.

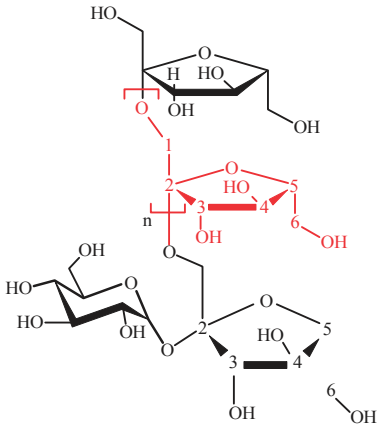

Fig. 5: Structure of LCP-2

The antioxidant activities of LCP-2 observed in this research are consistent with literature studies showing that fructans have robust antioxidant activities[49].

Immuno-stimulatory activities of *L. chinensis* polysaccharides: Immuno-stimulatory activities of LCP-1 and LCP-2 were measured by treating RAW 264.7 cells with purified LCPs.

Findings of this research indicated that LCP-1 and LCP-2 displayed immuno-stimulatory effects as demonstrated by increase in the production of TNF-α and IL-6 as a function of polysaccharide concentration (Fig. 6). It is extremely important to note that the results presented in Fig. 6a demonstrated that LCP-2 displays better activity than LPS (positive control) with respect to the production of TNF-α. As could be seen from Fig. 6c and d, immuno-stimulatory activities of LCP-1 and LCP-2 increase sharply when the polysaccharide concentration is greater than 30 μg mL^{-1}. Excellent immuno-stimulatory activities have been observed for LCP-2 at 125 μg mL^{-1} as indicated by (i) More than 12 fold enhancement in the

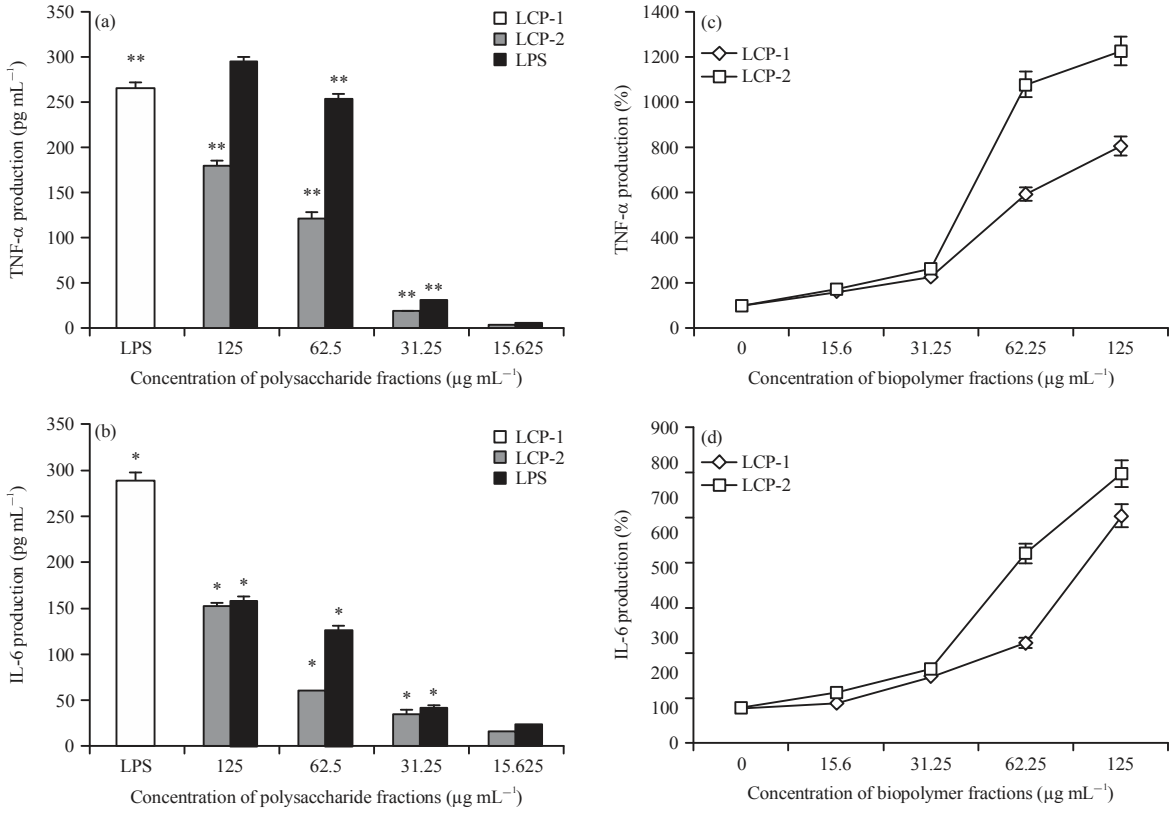

Fig. 6(a-d): Effects of *L. chinensis* polysaccharides on murine RAW 264.7 macrophages, (a, c) Production of tumor necrosis factor-α (TNF-α) and (b, d) Production of interleukin 6 (IL-6)

LPS was the positive control (100 ng mL^{-1}). ELISA assay was used for the quantification of IL-6 and TNF-α production, *Statistical difference for the positive control (LPS treated group) and the samples was significant, ($p<0.02$, $n = 3$), **Statistical difference for the positive control (LPS treated group) and the samples was significant, ($p<0.01$, $n = 3$)

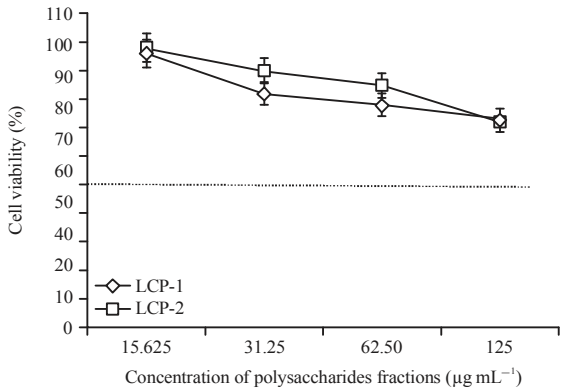

Fig. 7: Cell viabilities of isolated polysaccharides from *L. chinensis*

production of TNF-α in relation to the negative control (untreated macrophages) (Fig. 6c) and (ii) Nearly 8 fold increase in the production of IL-6 (Fig. 6d).

Cell viability: Effect of LCP-1 and LCP-2 on cell viabilities was given in Fig. 7. Results demonstrated that the polysaccharides from *L. chinensis* showed low toxicities even at the largest concentrations (125 μg mL^{-1}) studied in this research (Fig. 7). These findings are in agreement with those reported in the literature that plant polysaccharides display less toxicity[14,17,19-21,28-31].

The results demonstrated that *Lobelia chinensis* polysaccharides exhibit significant radical scavenging activities. An important point to be noted here is that LCP-2 is the major polysaccharide fraction present in this herb and the only mono-saccharide in this fraction is fructose. The antioxidant activities of LCP-2 observed in this research demonstrated the robust nature of antioxidant activities of fructans[49].

Findings of this research demonstrate that LCP-2 exhibited excellent immuno-stimulatory activities (Fig. 6c, d) and the observed activity is superior to the activity of positive control (LPS) with respect to the production of TNF-α. These observations are very significant and demonstrate that LCP-2 is highly suitable herbal polysaccharide to stimulate the immune system. Literature demonstrates that fructans can stimulate immune cells by binding to Toll like Receptor (TLR)[49]. It is important to note from the literature that the immunomodulatory activity plays a critical role in anticancer activity[14,17,19-21,28-31]. It is therefore concluded that the major component of *L. chinensis* polysaccharides (LCP-2) is highly potential candidate for immuno-chemotherapy. FT-IR and NMR spectroscopic studies have confirmed the structure of this fructan.

CONCLUSION

In this study, two polysaccharide fractions were isolated from *L. chinensis* (LCP-1 and LCP-2). A novel immuno-stimulatory polysaccharide (LCP-2) with β-D-(2→1)-fructofuranoside structure has been identified from *L. chinensis*. LCP-1 and LCP-2 have shown highly significant immuno-stimulatory and antioxidant activities. Especially, LCP-2 has exhibited extremely high immuno-stimulatory activity and less toxicity demonstrating that it has huge potential for immuno-therapeutic applications. This is a significant result reporting the isolation of an immuno-stimulatory fructan for the first time from *Lobelia chinensis*. These findings suggested the potential of *L. chinensis* polysaccharides to develop effective formulations for immunotherapeutic and anticancer applications.

SIGNIFICANCE STATEMENT

Lobelia chinensis Lour is an important anticancer herb used in traditional Chinese medicine. Abundant literature demonstrates that plant/herbal polysaccharides display significant immunomodulatory and anticancer effects with minimal toxicity. This research was aimed to isolate pure polysaccharides from *L. chinensis* and study their biological activities with a view to discover strong immunostimulators. The results of this research suggested that the polysaccharides isolated from *Lobelia chinensis* Lour were highly potential candidates for immuno-chemotherapy. Especially, one of the fraction (LCP-2) is a fructan that displayed extremely high immunomodulatory activity that was superior to the activity of LPS (a known positive control). The structure of this most active fraction (LCP-2) was found to be (2,1)-β-fructan. Major significance of this research is the discovery of immune-enhancing fructan from *L. chinensis.* This polysaccharide has huge potential to develop novel formulations for immunotherapy that will aid the discovery of effective immuno-chemotherapeutic regimen to treat cancer. Cancer therapy is expensive and many patients cannot afford the cost. Hence, the discovery of novel therapeutics from medicinal herbs will provide great benefit.

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