Original Article

Structural Characterization and Immunomodulatory Activities of Modified Pectic Polysaccharide from *Decalepis hamiltonii* (Swallow root)

Sathisha Upparahalli Venkateshaiah[1], Harish Nayaka M Annaiah[3], Harish Prashanth[2] and Shylaja Mallaiah Dharmesh[1*]

[1]Department of Biochemistry and Nutrition, Central Food Technological Research Institute, Mysore- 570020 [2]Department of Meat Fish and Poultry Technology, Central Food Technological Research Institute, Mysore- 570020 [3]Department of Sugar Technology, University of Mysore, Mysore- 570006

*Corresponding Author: Shylaja M Dharmesh, PhD Email : cancerbiolab@yahoo.co.in

Abstract

Decalepis hamiltonii (Swallow root) has been used extensively for medicinal properties in Ayurveda, the ancient Indian system of medicine. In the present study, we isolated pectic polysaccharides from *D. hamiltonii* root and for better biological activities, polysaccharides were modified using acid precipitation method. The Modified Swallow Root Pectic Polysaccharide (MSRPP) had higher polyuronic content and lower neutral sugar content. The MSRPP contains main neutral sugars were arabinose and galactose and homogeneity and revealed to be as arabinogalacturonan type having galacturonic acid and arabinose back bone with galactose branching analyzed by FT-IR, GC-MS and NMR analysis. The effects of MSRPP on murine macrophages (RAW) demonstrated potent immunomodulatory activity, induced production of reactive oxygen species, nitric oxide, TNFα, and IL-6. Furthermore, modulation of macrophage function by MSRPP was mediated through activation of NFκB. Together, our results showed that swallow root enriched source of varieties of bioactive compounds and MSRPP as an immunotherapeutic adjuvant and anti-metastatic activities.

Key words: Swallow root, Pectic Polysaccharides, Macrophage, Reactive oxygen species, nitric oxide, immunomodulatory.

Introduction

Decalepis hamiltonii (Wight and Arn.) is a climbing shrub of the family *Periplocaceae* (previously under *Asclepiadaceae*). It grows largely in moist as well as deciduous forests of Peninsula and southern parts of India in the hilly areas of Western Ghats (Gamble and Fischer, 1957). It is commonly called as Makali ber in kannada (native), whereas in English it is named as swallow root. Swallow roots are being used extensively for medicinal properties in Ayurveda, the ancient

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Indian system of medicine. Earlier reports have shown that swallow roots contain aldehydes, ketones, terpenes derivatives, alcohols, ketones (Murti and Seshadri, 1941a). Many studies have shown that *D. hamiltonii* possesses antioxidant properties and many bioactive compounds have been isolated and characterized (Harish et al. 2005; Srivastava et al. 2006). Having medicinal importance, the plant has been studied thoroughly for its pharmacognostical and phytochemical potential (Shefali et al. 2009; Nayar et al. 1978).

Pectins are the important structural components of the cell walls of plants. Of all the cell wall polysaccharides, pectins have the most complex structure. Pectins can constitute as much as 1/3 of the dry weight of the plant and are found in some plant juices (Kerry Hosmer Caffall, 2009). The molecular weight of pectins generally ranges from 25,000 to 400,000 Daltons. Essentially, pectins contain a "smooth" backbone of rhamnogalacturonan, which includes highly substituted rhamnogalacturon units (Ridley et al., 2001). Many chemical methods have been reported for the isolation of pectic polysaccharides from the cell wall. Extraction with chelating agents resulted in high molecular weight pectic polysaccharides, but of low yields (Zykwinska et al., 2006). Acid extraction is the most widely used method to obtain commercial pectin; however, it causes neutral side chain degradation (Levigne, Ralet, & Thibault, 2002). Alkaline extraction of pectic polysaccharides releases intact RG I including its neutral side chains through extensive hydrolysis of HG region by b-elimination and oxidative peeling (Zykwinska et al., 2006).

Among the dietary carbohydrates- pectic polysaccharides have been shown to play major therapeutic roles against cancer (Olano-Martin et al., 2003). Carbohydrates have an enormous potential for encoding biologic information. The biological activities of polysaccharides are influenced by their different solubility in water, molecular weights, degrees of branching and their different triple helical conformations (Nangia-Makker et al., 2002). Modified forms of pectic polysaccharides also have been shown to play significant therapeutic roles against cancers (Kidd, 1996) and in immunomodulation (Wong et al., 1994). The suggested hypothesis for modified pectin is that the structural elements that can bind and inhibit the functions of the lectin protein galectin-3, which is known as one of the diagnostic marker and a targeted protein for cancer metastatic

treatment (Takenaka et al., 2004, Platt and Raz 1992, Pienta et al., 1995).

D. hamiltonii roots are being used in Ayurveda, the ancient Indian system of medicine to stimulate appetite, relieve flatulence and also used as a general tonic. The roots are also used as a substitute for *Hemidesmus indicus* in Ayurvedic preparations. Earlier reports from our group and others have proved that swallow root is one of the enriched sources of varieties of bioactive compounds exhibiting bioactivities such as antioxidant, antimicrobial, anticancer, antiulcer, hepatoprotective and insecticidal properties (Srikanta et al., 2007; Sathisha et al., 2007; Naik et al., 2007; Murthy et al., 2006; Srivastava and Shivanandappa, 2006; Srivastava et al., 2006; Harish et al., 2005; Thangadurai et al., 2002; Harish Nayaka et al., 2010). The objective of this study is to investigate the structural properties of modified pectic polysaccharide from Swallow root by FT-IR, GC-MS and NMR analysis. In addition on Modified swallow root pectic polysaccharide (MSRPP) was subjected to immunomodulatory activities.

Materials and Methods

Chemicals
Carbohydrate standards such as rhamnose, arabinose, xylose, mannose, amberlite IR 120 H$^+$ resin, ammonium oxalate, galactose and glucose, protease, glucoamylase, thermoamylase, sepharose CL-4B, DMSO, D_2O, Sep-Pak C18 cartridge, KBr, N-cyclohexyl-N1-2-morpholinoethyl-carbodiimide-methyl-ptoluenesulfonate, Methyl iodide were purchased from Sigma Chemical Co. (St. Louis, MO, USA). OV-225 (1/8"x6') on Chromosorb W (80-100 mesh), SP-2330 capillary column (30 m X 0.32 mm i.d, 0.02m film coating) were purchased from Pierce Chemical Company, Rockford, USA. Other chemicals such as sodium phosphate buffer, acetic acid, sodium acetate, sodium chloride, triphenyl methane, sulphuric acid, Sodium hydride, acetonitrile, methanol, alcohol, Sodium borohydride and solvents used were of the analytical grade purchased from local chemical company. All chemicals and solvents used for analysis were HPLC grade.

Collection of sample
Fresh *D. hamiltonii* roots were purchased from a

local market; washed in water thoroughly, chopped into small pieces, air-dried and powdered to a particle size of 20 mesh. The powder was defatted in a soxhlet apparatus using hexane in the ratio of 200 mL/g (v/w). The defatted powder was air dried and preserved in dry condition under desiccation at 20° C until further extraction of pectic polysaccharides.

Isolation of a pectic polysaccharide.

Pectic polysaccharides were isolated following the ammonium oxalate extraction method (Phatak et al., 1988) as depicted in the Scheme 1. Briefly, 10 g of defatted powder was depleted with proteins, amylose and amylopectins by specific enzymatic (protease, termamylase and glucoamylase) digestions at their optimum reaction conditions and centrifuged. Further, the residue was extracted with 200 ml of 0.25% (w/v) ammonium oxalate solution and filtered; the filtrate was precipitated by ethanol at 4° C. The precipitate was re-suspended in 10 ml of water and lyophilized to obtain pectic polysaccharide and designated as Swallow root pectic polysaccharide (SRPP).

Small molecular weight forms of the pectic polysaccharides were prepared by acid precipitation method as described by Pienta et al., 1995. Briefly pectic polysaccharide was solubilized as a 1.5 % (w/v) solution in distilled water, and its pH was increased to 10.0 with 3N NaOH for 1 h at 50-60° C. The solution was then cooled to room temperature while its pH was adjusted to 3.0 with 3N HCl and stored overnight. Samples were precipitated the next day with 95 % ethanol and incubated at –20° C for 2 h, filtered, washed with acetone, and dried on Whatman No.1 filter paper. Polysaccharides obtained were designated as modified Swallow root pectic polysaccharide (MSRPP).

Gel permeation chromatography separation and molecular weight (MW) determination.

Gel permeation chromatography was performed for potent intact SRPP as well as modified forms of pectic polysaccharides (MSRPP) on Sepharose CL-4B column (1.6 cm x 92 cm). The elution was carried out using NaCl (0.1 M) containing sodium azide (0.05 %) at a constant flow rate of 16 mL/h. Fractions (3 mL) were collected and analyzed for the presence of total sugar, and appropriate fractions were pooled. The column was pre-calibrated with standard dextran molecular weights T-series. A calibration curve was prepared by plotting Ve/Vo (elution volume/void volume, mL) vs log MW and MW of the unknown

polysaccharide were determined (Fihman ML et al., 1984). Molecular weights (MW) of the polysaccharide of intact (SRPP) and modified forms (MSRPP) were determined from the calibration graph.

Determination of total carbohydrate content

Total sugars were estimated by the phenol-sulphuric acid method (Pragna Rao et al., 1989). Sugar content was determined against the calibration graph, prepared by using D-glucose at 4-20 µg/mL range.

Sugar composition analysis by GLC method

Sugar composition in the fractions was determined by GC (Shimadzu, Model CR4A) after hydrolyzing the samples and converting the hydrolysates to alditol acetates (Raju et al., 2001).

Uronic acid estimation

The toatal uronic acid were estimated by the carbazole method (Bitter and Muir, 1962). Uronic acid content was determined against the calibration graph prepared using D-galacturonic acid at 10-50 µg/mL concentration range.

Fourier Transform-Infrared spectroscopy (FT-IR)

The SRPP/MSRPP samples (6 mg), in duplicates was mixed thoroughly with solid crystalline KBr (Spectroscopic grade) and pressed into a 1 mm pellet (Manuel et al. 1998). IR spectral studies were performed in an absorbance mode at a resolution of 4 cm^{-1} with wave number range 400-4000 cm-1 using a Perkin Elmer spectrum 2000 spectrometer (Connecticut, USA).

Methylation analysis

Polysaccharides (10 mg) were methylated by following the method of Hakomori (1964). Permethylated polysaccharides were hydrolyzed with formic acid and sulfuric acid, successively, acetylated and the partially permethylated alditol acetates were analyzed by GLC–MS. GLC–MS analysis was performed on a Shimadzu (Model QP 5000) using a SP-2330 capillary column (ɸ 0.31 mm × 30 mm) operating at an ionization potential of 70 eV with a temperature programme (180–200° C, 4° C rise per min). Chloroform was used as solvent and the carrier gas was helium.

NMR spectral analysis

The MSRPP sample was dissolved in 1 mL of D$_2$O,

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spectra were recorded with a Bruker amx 400 spectrometer at 500/700 mHz.

2.11. Immunomodulatory activity:
2.11.1. Cell Culture
RAW 264.7 macrophage cells were cultured in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% FBS (Hyclone), penicillin (100 U/mL), and streptomycin (100 µg/mL) (Invitrogen) and incubated at 37° C with 5% CO_2 condition.

2.11.2. Nitric oxide production
Nitrite in the cellular media was measured by the Griess method (Morihara et al., 2002). RAW cells were cultured in the presence of different concentrations of MSRPP (100 and 200 µg/mL) in a 24-well microtiter plate (5×10^5 cells/well) in a total volume of 1 mL. After 48h stimulation, the cellular media were collected in the end to assay for nitrite. 50µl of cell-free supernatant, incubated with 50µl of Griess reagent (1 % sulfanilamide, 0.1 % naphthylenediamine dihydrochloride, 0.5% H_3PO_4) at RT for 5 min and the absorbance at 550nm. The concentration of nitrite (µM) was calculated using a standard curve produced from a known concentration of sodium nitrite. The results are presented as the means ± standard deviations (SDs) of four replicates of one representative experiment.

### 2.11.3 Determination of TNF-α, IL-1β, NF-κB and IFN-γ
The RAW cells (5×10^5 cells/well) were incubated in a 24-well tissue culture plate with standard culture medium in the absence (control) or presence of MSRPP (200 µg/ml). LPS (5µg/ml) was used as a positive control. After 48 h, the supernatant was collected and preserved at −70° C. The IL-1β, IFN-γ, NF-κB and TNF-α concentrations in the supernatant were detected by enzyme-linked immunosorbent assay (ELISA) (R&D Systems) according to the manufacturer's protocol. The results are presented as the means ± SDs of three replicates of one representative experiment (Byun, Ryu, & Lee, 2006).

3. Results
3.1. Isolation of pectic polysaccharides from dietary sources
Pectins are polymers commonly found plant foods and contain potentially abundant levels of galactose.

Pectic polysaccharides from *D. hamiltonii* were isolated by the ammonium oxalate extraction method which yielded 4 %. Further, the molecular weight of SRPP being higher, upon controlled hydrolysis using acid precipitation procedure, 80 % of the polysaccharide as MSRPP was recovered (**Scheme 1**) (Sathisha U.V., 2007).
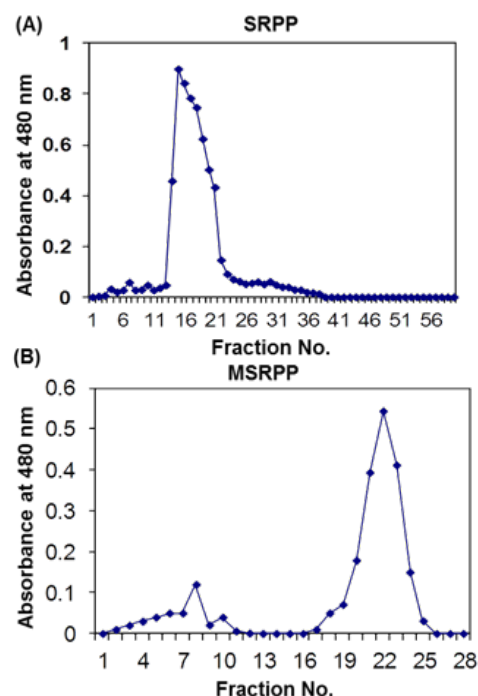
Figure 1. High-performance size exclusion chromatograms (HPSE) of SRPP and MSRPP: Separation of Swallow root pectic polysaccharide (SRPP), Modified Swallow root pectic polysaccharide (MSRPP) (A & B) on Sepharose CL – 4B column. Total carbohydrate was determined by phenol-sulfuric acid assay at 490 nm.

3.2. Determination of Molecular weight by Gel permeation chromatography
To determine the purity and relative molecular weight of the modified form of SRPP, MSRPP was subjected to Sepharose CL-4B column chromatography, calibrated with standard dextrans and the respective elution volume (Ve) was determined. Results of chromatography indicated that MSRPP yielded two peaks constituting 15 % of peak 1 resembling the native SRPP (**Fig.1a**) with Ve ~ 32 mL and 75 % eluting at Ve 62 mL corresponding to the molecular size of ~ 50 kDa (**Fig.1b**). Calculated change in the elution

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volume of MSRPP relative to that of SRPP indicated the reduction in the size of the polysaccharide. Large molecular weight of SRPP - 800 kDa has been reduced to ~ 50 kDa.

3.3. Sugar composition analysis

The sugar composition analysis of *D. hamiltonii* polysaccharide (SRPP and MSRPP) was determined by GLC. Sugar composition analysis as in **Table.1** revealed the presence of rhamnose: arabinose: xylose: galactose in the percent ratio of 14:35:3:48.

3.4. FT-IR analysis of MSRPP

The FT-IR spectral analysis of MSRPP was performed employing an infrared spectrophotometer. **Fig. 2A** depicts the IR spectral profile of MSRPP in the frequency of 400-4000 cm^{-1}. It showed the characteristic groups of a pectic polysaccharide. Absorption at 3408 cm^{-1} indicates the presence of free –OH groups, CH stretching at 2919 cm^{-1} associated with ring carbon atoms, skeletal mode absorption of the glycosidic linkage at 950 cm^{-1}, the absorption at 1014 cm^{-1} corresponded to galacturonic acid residues. Band at 1737 cm^{-1} is indicative of an ester carbonyl group stretch. CH2 stretching band at 2930 cm^{-1}, while absorption at 1616 cm^{-1} indicates the presence of C=O (Carboxylic group). Absorption at 831 cm^{-1} depicts the β-conformation, signals at spectral region of 800-1200 cm^{-1} could be due to highly coupled c-c-o, c-H and C-O-C stretching modes indicative of the polymer backbone. IR spectral analysis thus indicates the nature of MSRPP as polymer with uronic acid residues. Uronic acid is indeed a component of pectic polysaccharide.

3.4. Structural elucidation of MSRPP by GC- MS

The structural aspects of pectic polymers have been reported in fruits like apple (Schols et al., 1995). Most of these studies on pectins are based on the FT-IR, GLC analysis, ^{13}C NMR andmethylation studies followed by GC-MS. Combinations of methods were used for structural analysis of MSRPP.

3.4.1. Reduction of Carboxyl groups

The uronic acid content in MSRPP was 162.6 mg/g and subjected to the carboxyl reduction using carbodiimide and sodium borohydride, carboxyl

groups were reduced to 5%. Methylation study has been widely used for linkage analysis of the pectic polysaccharides (Schols et al., 1995). In order to elucidate the linkage and substitution pattern, carboxyl reduced MSRPP was permethylated and the derivatives were analyzed by GC-MS on SP-2330 column. $2.3.4.6-Me_4$ glucose was used as a reference standard for determining relative retention time (**Fig. 2B**). Diagnostic fragments of samples in **Table 2** showed about 69.2 % of $2.3.6 \; Me_3$ galactose and 24.5% of $2.3 \; Me_2$ arabinose. Arabinose and galactose appear to be in the backbone of the pectic polysaccharide with 1→4 linkage. The galacturonic acid content was 162.6 mg/g, the back bone of the MSRPP could be a galacturonic acid polymer in 1→4 linkage. The presence of $2.3. \; Me_2$ galactose indicates branching of the polysaccharide. $2.3.4.6. \; Me_4$ galactose indicated terminal galactose. Arabinose was present as $2.3. \; Me_2$ arabinose and would arise from the 1→5 linked chains present as side chains and may be in furanose
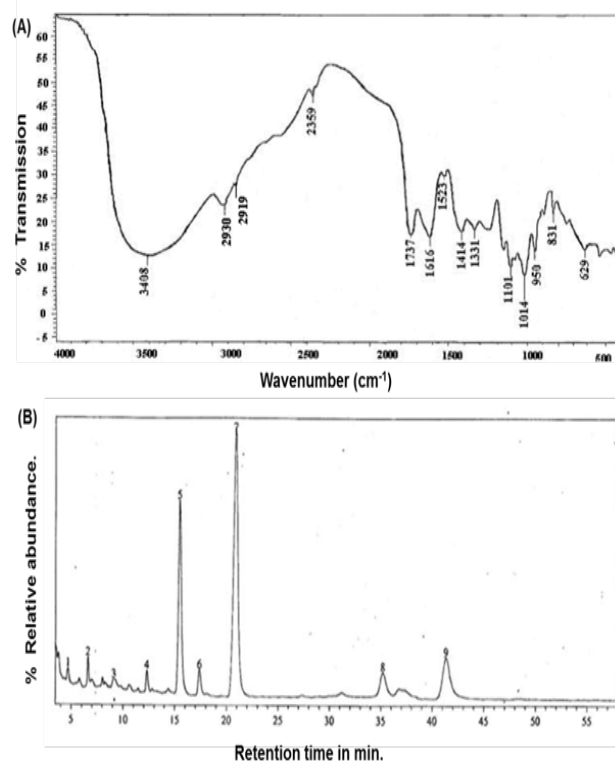
Figure 2. Fourier transform infrared spectroscopy spectra FT-IR Spectroscopic profile (A) of MSRPP of pectic samples and GLC profile (B) of permethylated of MSRPP pectic samples to detect functional groups.

ring form. Rhamnose was present as 3.4. Me2 rhamnose indicating chains of rhamnose with 1→2 linkage. Rhamnose and arabinose would also be attached to galacturonic acid backbone. Arabinogalacturonans containing side chains composed of arabinose and galactose are reported

Table 1. Relative percent sugar composition analysis content of SRPP and MSRPP

Sugars	SRPP	MSRPP
Rhamnose	16	14
Arabinose	50	35
Xylose	02	03
Mannose	-	-
Galactose	32	48
Glucose	-	-
Uronic acid in mg/g	141.3	162.6

from kiwi fruits (Redgwell et al., 1991). Based on the consolidated results of structure analysis, tentative structure of MSRPP has been proposed in **Fig. 3**. MSRPP was revealed to be as arabinogalacturonan type having galacturonic acid and arabinose backbone with galactose branching. It also had branches of rhamnose and arabinose intern attach to galactose at terminal positions.

3.4.2. NMR Analysis of MSRPP
Structure proposed for MSRPP was substantiated by NMR analysis. ^{13}C NMR Spectrum of MSRPP depicted in **Fig. 4A**, reveals signals characteristic of a pectic polysaccharide, thus confirming that the isolated molecule is a pectic polysaccharide. The main β 1-4 linked –galacturonic acid units were characterized by six signals at 99.38, 80.97, 71.7 and 72.6. Signals at 60.17 and 69.2 can be assigned to ring carbons of C-1, 4, 3, 5, 2 & 6 respectively. A sharp signal at 99.18 ppm corresponds to C-1 ring anomeric due to β-conformation. The signal at 19.22 ppm is attributed to C-6 of β - rhamnose units. A split in the C-1 signal could be due to the anomeric carbons corresponding to galactose and galacturonic acid residues and is the primary constituents of the pectic polymer.

Table 2: Mass spectra and fragmentation scheme of partially methylated alditol acetates

Peak no	Retention time	O-Methyl ester	Fragmentation pattern	Mode of linkage
2	1.33	3.4. Me2 Rha	43, 57, 115, 131, 160, 175, 190, 234	-2)-Rha-(1-
4	0.96	2.3. Me2 Gal	43, 118, 129, 162, 233, 261, 305	-4.6)-Gal-(1-
5	24.5	2. 3 Me2 Ara	43, 118, 129, 162, 189, 205, 233	-5)Ara(1-
6	2.08	2.3.4.6. Me4 Gal	43, 45, 101, 118, 129, 162, 205, 249	Gal-(-
7	69.2	2.3.6. Me3 Gal	43, 118, 131, 162, 173, 203, 233, 277	-4)Gal-(1-
9	2.02	2.3.4. Me3 Gal	43, 118, 129, 162, 173, 189, 201, 233	-6)Gal-(1-

3.5. Immunomodulatory activity
3.5.1. Effect of MSRPP on macrophage NO production
To evaluate the role of MSRPP on NO production by the RAW macrophages, concentrations of 100-200 µg/ml were used (**Fig. 4B**). The nitrite concentration of the culture supernatant was significantly altered by 100-200 µg/ml MSRPP, and there was an increase of 44.5% in the NO
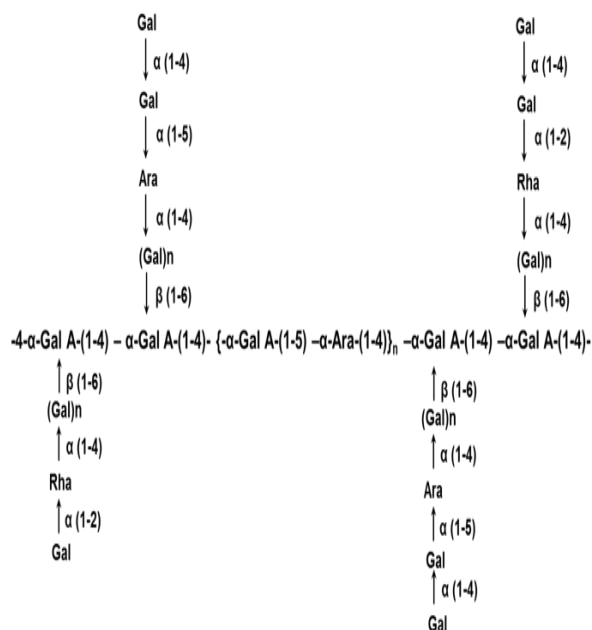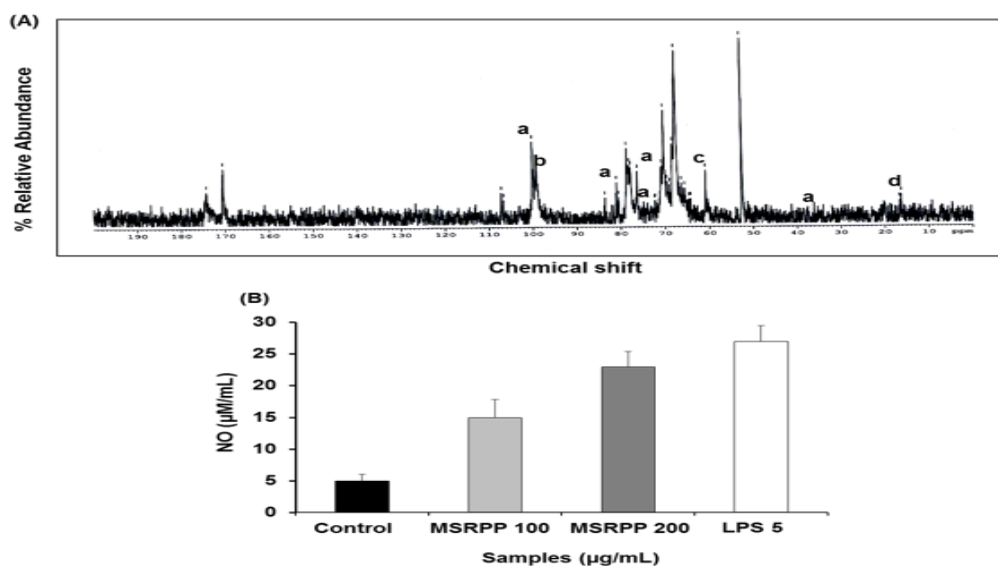

Figure 3. Tentative structure of the MSRPP of pectic samples.

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Figure. 4: NMR – Spectral chemical shifts (ppm) of MSRPP (A). Effect of MSRPP polysaccharides on RAW murine macrophage ROS (B). RAW macrophages were incubated for 24 hr with the indicated concentrations of MSRPP polysaccharides, or 5 µg/ml LPS (positive control). NO production was quantified by measuring nitrite in the cell-free supernatants. Values are the mean ± S.D. of triplicate samples from one experiment. Statistically significant differences (P<0.05) between untreated cells and cells treated with fractions are indicated.

at the concentration of 200 µg/ml compared to the negative control (medium). This suggests that, MSRPP may stimulate the NO production on RAW macrophage. Indeed, NO production induced by MSRPP was comparable to that induced by 5 µg/ml LPS **(Fig. 5).**

3.5.2. Effect of MSRPP on macrophage TNF-α, IFN-γ, NF-κB and IL-1β production

Immunomodulatory compounds can regulate cytokine production. We examine culture supernatants whether MSRPP activates cytokines on RAW cells; the culture supernatants were collected at 48h, and the amounts of TNF-α and IL-1β were measured by ELISA. Thus,
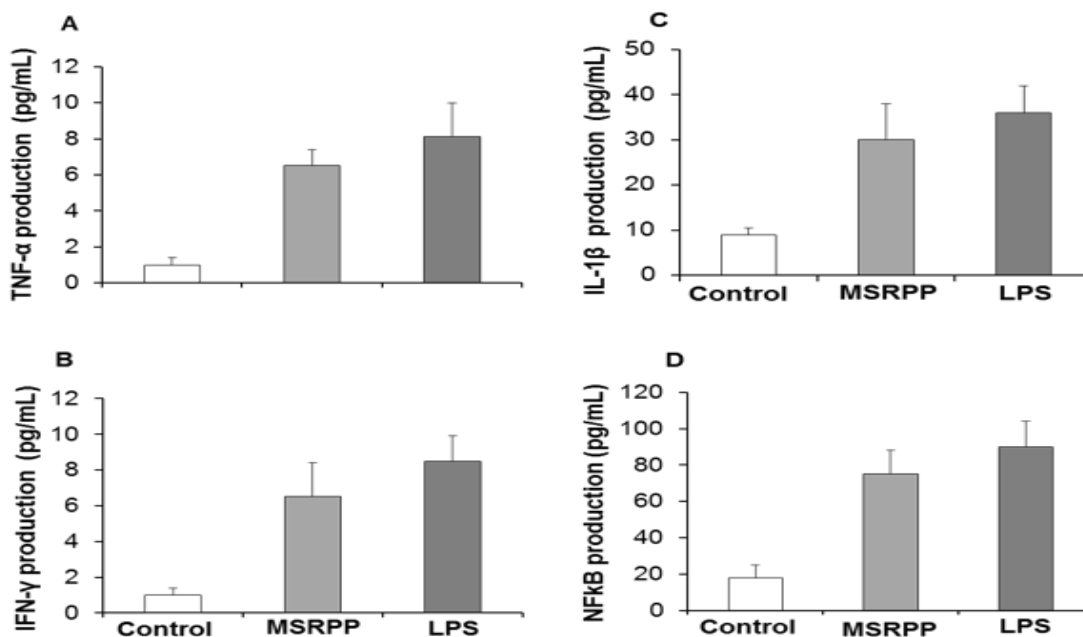
Figure 5: Effect of MSRPP polysaccharides on RAW murine macrophage IL-1β (A), TNF-α (B), IFN-γ (C) and NF-κB (D) concentrations in the supernatant were detected utilizing an ELISA assay according to the manufacturer's protocol. RAW macrophages were incubated for 48 h with the indicated concentrations of MSRPP polysaccharides, or 5 µg/ml LPS (positive control). The results are presented as the means ± SDs of three replicates of one representative experiment.

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we analyzed the effects of MSRPP polysaccharide fractions on macrophage IL-1β, IFN- γ and TNF-α production. Untreated murine RAW macrophages produced low cytokines; whereas, incubation of these cells with the MSRPP polysaccharide significantly enhanced IL-1β (20 pg/ml), IFN-γ (3.5 pg/ml) and TNF-α (25 pg/ml) production in a concentration-dependent manner (**Fig. 5**). These responses were quite strong, and the levels of TNF-α induced by MSRPP were comparable to those induced by LPS in murine RAW cells. Thus, these results indicate that MSRPP polysaccharides induce a significant up-regulation of TNF-α, IFN- γ and IL-1β synthesis in murine RAW macrophages. To evaluate signaling pathways involved in the immunomodulatory activity of MSRPP polysaccharides, we utilized a transcription murine RAW macrophages. To evaluate signalingpathways involved in the immunomodulatory activity of MSRPP polysaccharides, we utilized a transcription factor-based bioassay for NF-κB activation in murine RAW macrophages. MSRPP potently activates the NF-κB pathway at concentration of 60 pg/ml which even higher than that induced by 90 pg/ml LPS.

4. Discussion

The plant pectins have been known to help immune, and digestive health through their events in the gastro intestinal tract, the main barrier prevent them from exert systemic benefits all over the body has been their bioavailability. The pectins have too long complex and connection of metastatic melanoma cells to the liver parenchyma by aggressive binding to the liver hepatic galactose receptors, however larch arabinogalactan offers the additional benefit of enhancing immune function (Takenaka et al., 2004, Pientaet al., 1995).

Indeed the presence of arabinose and galactose appeared to be important for antimetastatic activity (Sathisha U.V, 2007). Larch wood arabinogalactan has a broad range of potential therapeutic applications including dietary fiber supplement, including the ability to improve the growth of friendly bacteria, increase the production of SCFAs, and decrease ammonia generation. Larch Arabinogalactan, as a biological response modifier also appears to offer substantial promise effects on NK cytotoxicity. Both immune-stimulating properties along with its anti-metastatic activities fulfill two therapeutically desirable goals.

Cytokines regulate both cellular and humoral immune responses by affecting immune cell proliferation, differentiation and functions. IL-2 has many immune potentiating effects, such as and connection of soluble fibers make it difficult to absorb into the circulation. This event was resolved with the progress of modified pectins like modified citrus pectins (MCP), prepared using a modification process to reducing the size and cross branching of citrus pectin (CP) (Pienta et al., 1995). MCP absorb into the circulation, and modification allow easily absorb into the circulation and use several therapeutic effects. While MCP has traditional attention in both conventional and alternative medical researchers for its anti-metastatic activity, has been recognized to be useful in the treatment and prevention of metastatic cancer, solid tumors like melanoma and prostate, colon, and breast. Many Scientists believe that MCP mechanism by inhibiting key progression involved in cancer development: angiogenesis and metastasis (Platt and Raz 1992). Larch arabinogalactan mechanism in the same pattern, by inhibiting the metastasis melanoma proliferation of T-cells, B-cells, NK cells and monocytes, augmentation of cytotoxicity of T-cells and NK cells and *in vivo* production of lymphokine-activated killer (LAK) cells, which exhibit high cytolytic activities against autologous tumor cells (Ehrke, M. J. 2003.). The surfaces of macrophage's array diverse receptors that may bind different polysaccharides and activate the cells to produce mediators, such as TNF-α and IL-1β (Commins et al., 2010). TNF-α can act on monocytes, and macrophage's in an autocrine manner to improve different functional responses and induce the expression of other immunoregulatory and inflammatory mediators (Baugh JA and Bucala R., 2002). IL-1β is involved in fever during the induction of the acute phase protein response. In addition, it cooperates with IFN-γ and IL-12 to induce tumor death by NK cells (Ross JA and Auer MJ., 2002). IFN-γ is an important immunoregulatory molecule (Blankenstein and Qin, 2003). It induces the production of T cells, activates macrophages, and regulates crossly Th1 and Th2 cells. IFN-γ and TNF-α can enhance immunoregulatory ability each other towards tumor.

Our results showed that MSRPP promoted a clear increase in IL-1β and TNF-α. The levels of

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TNF-α and IL-1β induced by 200µg/mL were comparable to those induced by LPS. This demonstrated that MSRPP could be used to trigger macrophages to activate antitumor activity. In the present work, a probable relation of NO dependant cytokine release was observed. MSRPP increased NO production and did not influence the production of superoxide anion. This result suggests that this polysaccharide could interact with Toll-like receptors and then activate NF-kB signaling pathway (Ando I, et al., 2002). Thus, the activation of the NF-kB increases NO release and triggers production of TNF-α and IL-1β (Han SB, et al, 2003).

5. Conclusion
Overall, our study concludes that MSRPP has been structurally characterized by FT-IR, GC-MS and NMR analysis. Modified pectic polysaccharide revealed as arabinogalacturonan type having galacturonic acid and arabinose backbone with galactose branching. In addition, branches of rhamnose and arabinose intern attach to galactose at terminal positions. Further, MSRPP polysaccharides showed a significant up-regulation of TNF-α, IFN- γ and IL-1β synthesis in murine RAW macrophages. Therefore, activation of proinflammatory cytokine production and bioactive principles of MSRPP lead a new insight as a therapeutic target and in drug discovery to inhibit metastatic tumor progression. Huge biological potential of *D. hamiltonii* root can be an effective anti-metastatic therapy, which needs to be, addressed in the future course of study.

6. Acknowledgement

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