



ISOLATION AND CHARACTERIZATION OF ANTIMETASTATIC COMPONENTS FROM BLACK CUMIN (*NIGELLA SATIVA*) AND SWALLOW ROOT (*DECALEPIS HAMILTONII*)

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Article Received on 11/10/2018

Article Revised on 31/10/2018

Article Accepted on 21/11/2018

ABSTRACT

Cancer is a complex multi-step disease, rising constantly in both developing and developed countries leading to mortality. Developing a strategy for cancer therapy has been difficult since cancer cell poses lots of challenges to normal cells by self-regulating their survival and dysregulating normal cellular action. Current study explores the possibility of dietary antimetastatic polysaccharides from black cumin (*Nigella sativa*) and swallow root (*Decalepis hamiltonii*) that can effectively target galectin-3, a key molecule in metastasis causing severity of cancer pathogenicity and death. The results showed that swallow root had higher yield of ~ 6.2% of pectic polysaccharide [SRPP] as opposed to 0.8% in Black cumin pectic polysaccharide (BCPP). Also, SRPP had ~ 14.6 and 70 fold higher galectin-3 inhibitory activity when compared to galectin specific sugar - galactose and BCPP, respectively, indicating the increased antimetastatic potency of SRPP. Purification of SRPP followed by activity determination indicated that SRPP – 0.15 M fraction was more active with ~ 462 fold increase in activity than that of the crude SRPP; Active fraction had a molecular size of 250 kDa. Sugar composition analysis and correlation studies suggested that precise arrangement of arabinose and galactose in an arabinogalactan pectic polysaccharide is important in offering potent galectin-3 inhibitory effect. Galectin-3 inhibitory effect could be attributed to antimetastatic activity.

KEYWORDS: Cancer is a complex multi-step antimetastatic activity.

INTRODUCTION

Diet contains carbohydrates of various size, concentrations and chemical sequences starting from simple monosaccharides to complex polysaccharides. Among them pectic polysaccharides in particular has been shown to play critical therapeutic roles against cancer (Olano-Martin *et al.*, 2003), immunomodulation (Wong *et al.*, 1994), ulcer (Kiyohara *et al.*, 1994) etc.

Pectic polysaccharide also called pectin is a linear chain of α -(1-4)-linked D-galacturonic acid that forms the pectin-backbone, a homogalacturonan. Into this backbone, there are regions where galacturonic acid is replaced by (1-2)-linked L-rhamnose. From rhamnose, sidechains of various neutral sugars branch off. This type of pectin is called rhamnogalacturonan I. Over all, up to every 25th galacturonic acid; in the main chain is exchanged with rhamnose. Some stretches consisting of alternating galacturonic acid and rhamnose – “hairy regions”, others with lower density of rhamnose –

“smooth regions”. The neutral sugars are mainly D-galactose, L-arabinose and D-xylose; the types and proportions of neutral sugars vary with the origin of pectin. Often sugars are also found derivatized as - methylated (a), amidated (b) etc.

Recently the role of pectic polysaccharides has gained importance due to their role played in controlling cancer metastasis through the blockade of galectin present on the metastatic cancer cells (Inohara & Raz, 1994) as galectin-3 expression is correlated with metastatic potential in certain malignancies (Bresalier *et al.*, 1997). Results of several investigations have revealed the possibility of galectin-3 as a diagnostic marker in certain cancers and also one of the target proteins for cancer treatment (Konstantinov *et al.*, 1996). Further higher levels of galectin-3 have been shown to correlate with the advancement of the cancer disease and it is believed that galectin-3 of cancer cells bind to normal cells and establishes secondary tumors. Galectin-3 hence has been

implicated in tumor spread and metastasis (Takenaka *et al.*, 2004). Studies have also indicated that oral administration of modified citrus pectin reduced the rate of cancer cell spread and inhibited metastasis in animal models (Pienta *et al.*, 1995). Despite promising role of dietary pectins against metastasis, hardly few reports are available on identification of such components from dietary sources, which can potentially inhibit the binding of galectins to α -D-galactoside residues present on the extracellular matrix components of normal cell surface as well as to the basement membranes, thereby preventing cancer spread. We designate such components as antimetastatic components (AMC).

In the present study, efforts were made to isolate pectic polysaccharides from swallow root (SRPP) and black cumin (BCPP) and studied their antimetastatic potential. Purification and characterization studies were also undertaken for swallow root pectic polysaccharide which was significantly active. Sugar composition analysis however was compared between BCPP and SRPP to understand the probable composition required for the activity.

MATERIALS AND METHOD

Chemicals

Minimum essential medium (MEM), Dulbecco's minimum essential medium (DMEM), glutamine, sodium bicarbonate, penicillin, kanamycin, F12 mixture and heat inactivated fetal calf serum (FCS), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide (MTT), 4-(2-hydroxyethyl) piperazine-1-ethanesulfonic acid (HEPES), sucrose, ethylenediaminetetraacetic acid (EDTA), triton X 100, tween 20, skimmed milk powder, paranitrophenyl phosphate (PNPP), diethanolamine, Alsever's medium, trypsin, hematoxylin, eosin, acridine orange, carbohydrate standards such as rhamnose, arabinose, xylose, mannose, galactose and glucose, protease, thermoamylase, glucoamylase, were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Sepharose CL-4B (4% cross-linked, fractionation range for dextrans 30,000-50,000 Da), DEAE-cellulose (0.99 meq/g), Amberlite IR-120-P (8% cross-linked, 16-50 mesh), Dextran standards, T-Series viz., T-10, T-20, T-40, T-70, T-150, T-500, T-2000 were obtained from Pharmacia Fine Chemicals, Uppsala, Sweden. Alkaline phosphatase conjugated – rabbit anti mouse IgG secondary antibody was procured from GENEI, Bangalore, India. HPLC column (Shimpak C18) was obtained from Shimadzu Corp. Tokyo, Japan. 3% OV-225 (1/8" $\times$ 6') on Chromosorb W (80-100 mesh) was from Pierce Chemical Company, Rockford, USA. Other chemicals such as hexane, ammonium oxalate, iodine solution, sodium phosphate buffer, perchloric acid (HClO₄), acetic acid, sodium acetate, glutaraldehyde, glycine, sodium chloride, sulphuric acid and solvents used were of the analytical grade purchased from local chemical company. All chemicals and solvents used for HPLC and GLC were HPLC grade.

Isolation of pectic polysaccharides

Fresh swallow root and black cumin, were purchased from a local market (Devaraja Market, Mysore, Karnataka, India), while citrus pectin was procured from Sigma Chemical Co, USA. Fresh swallow roots were chopped into small pieces and air dried in the dark in a ventilated hood. The air dried samples of swallow root and black cumin were ground and defatted in a soxhlet apparatus using hexane (200 mL/g, w/v). The defatted powder was air dried and preserved in dry condition until further extraction of pectic polysaccharides.

Pectic polysaccharides were isolated following the ammonium oxalate extraction method (Phatak *et al.*, 1988). Briefly 100 g defatted sample was treated with 100 mg of protease in 500 mL of 0.1 M sodium phosphate buffer (PB) of pH-7.5 and incubated overnight at 37 °C with stirring. Contents were filtered and the filtrate was discarded after successive washing-3 times with PB. The residue was suspended in 500 mL of PB along with thermoamylase (100 mg) and boiled for 15 min. After ensuring complete degradation of starch with iodine solution, the contents were filtered and supernatant was discarded. The residue was resuspended in acetate buffer (0.1 M, pH-4.5) along with glucoamylase (100 mg) and incubated overnight. Further, the contents were filtered and the residue was taken in 200 mL ammonium oxalate (0.25% w/v, pH-3.5) solution and boiled for 1 h at 70 °C with occasional shaking. The contents were filtered and the supernatant was precipitated with 4 volumes of absolute ethanol and kept in cold for 1 h. Contents were centrifuged at 5000 *g* at RT for 20 min and the pellet was washed twice with 50 mL ethanol (45%). Finally, the pellet was resuspended in 100 mL of water, dialyzed extensively against water and lyophilized to get pectic polysaccharide. In order to confirm that the isolated fraction is a pectic polysaccharide sugar composition analysis and uronic acid contents were determined since it is the characteristic sugar component of pectic polysaccharide.

Total carbohydrate estimation

Total carbohydrate content was estimated (Dubois *et al.*, 1956) in 0.5 mL of the sample (10 mg/100 mL of water) in a test tube. To samples, 0.3 mL of phenol (5%) and 1.8 mL of concentrated sulphuric acid were added and the contents were mixed thoroughly. After cooling the tubes at room temperature (~20 min), the absorbance was read at 480 nm against a reagent blank. Sugar content was determined against the calibration graph, prepared by using D-glucose (4-20 μ g/mL).

Uronic acid estimation

Uronic acid was estimated (Bitter & Muir, 1962) in 0.5 mL of the sample solution (10 mg/100 mL) in a test tube and kept in ice cold water bath for 10 min. To this was added concentrated sulphuric acid (3 mL) slowly, contents were mixed thoroughly and kept in boiling water bath for 20 min. Contents were cooled to which

carbazole solution (0.1 mL, 0.1% prepared by dissolving re-crystallized carbazole in alcohol) was added. The tubes were kept in dark for 2 h and the absorbance was recorded at 530 nm. Uronic acid content was determined against the calibration graph prepared by using D-galacturonic acid (10-50 µg/mL).

Fractionation of Pectic polysaccharides

DEAE cellulose column chromatography

DEAE-cellulose was washed with water to remove fine particles. It was then regenerated successively with HCl (0.5 N) and NaOH (0.5 M). After each treatment, the pH was adjusted to neutrality by washing thoroughly with water. The regenerated exchanger was suspended in ammonium carbonate (0.5 M, pH 9.3), packed in a column (3.5 cm x 26 cm) and excess carbonate was washed off with water.

DEAE-cellulose column and the elution was carried out with water, followed by ammonium carbonate (0.05 to 0.20 M) and sodium hydroxide (0.1 and 0.2 M) solutions. The flow rate was maintained at 60 mL/h and fractions (10 mL) were collected, assayed for total sugar by phenol-sulfuric acid method as described earlier. Carbohydrate positive fractions were pooled, dialyzed and lyophilized. Fractions – 0.01 M, 0.1 M, 0.15 M, and 0.2 M were examined for galectin-3 inhibitory property in composition with the crude SRPP and active fraction was further characterized. Fractions were designated as SRPP - 0.05 M, SRPP – 0.1M, SRPP – 0.15 M and SRPP - 0.2M respectively.

Growth and maintenance of MDA-MB-231 cells

MDA-MB-231 is a metastatic human breast adenocarcinoma was procured from National Centre for Cell Sciences, Pune, India and maintained in the animal cell culture laboratory at CFTRI. Cells were grown in DMEM-high glucose (4.5 g/L) with 4 mM glutamine, 1.5 g/L sodium bicarbonate, penicillin (100 units/mL) and kanamycin (0.1 mg/mL) with 10% fetal calf serum at 37 °C in a humidified chamber with 95% air and 5% CO₂. Cultured cell growth and survival was monitored by MTT assay (Hansen *et al.*, 1989). Cells (2.8×10⁴ cells/mL) were cultured with the specified medium in a 96 well microplate and after 72 h, 25 µL of MTT solution (5 mg/mL) was added, incubated at 37°C for 4 h. One hundred micro litre of lysis buffer was added and cells were continued to incubate at 37°C overnight (about 16 h) to dissolve the dark blue crystals and absorption of formazan solution was measured at 570 nm in a microplate reader (Spectra Max-340, Molecular Devices, Germany). Presence of galectin-3 was measured employing monoclonal antibody specific to galectin-3 by ELISA as described by Sathisha *et al.*, 2007. Of the several cell lines screened MDA-MB-231 was expressing the highest level with 15-fold higher over a Normal - NIH-3T3- a Mouse embryonic fibroblast cells. Media of MDA-MB-231 cells after 48 h of growth, also showed higher levels of galectin and this has been used in *in vitro* to study galectin-3 mediated

agglutination of red blood cells, while evaluating the galectin- inhibitory pectic polysaccharides as a measure of antimetastatic property.

Agglutination inhibition assay

Microplate agglutination assay was performed for the evaluation of potential dietary galectin inhibitors (antimetastatic components) employing the protocol of Nowak *et al.* (1976). Briefly, human erythrocytes were prepared from 10 mL of fresh blood (collected in Alsever's medium), washed four times with five volumes of 0.15 M NaCl. A 4% erythrocyte suspension in 0.02 M PBS, pH 7.4 containing 1 mg/mL trypsin was incubated for 1 h at 37°C. The trypsin treated cells were washed with five volumes of 0.15 M NaCl and fixed in five volumes of 0.02 M PBS pH 7.4 containing 1% glutaraldehyde for 1h at room temperature. Glutaraldehyde fixation was terminated by the addition of five volumes of 0.1 M glycine in PBS, pH 7.4 at 4°C and the fixed erythrocytes were employed for the hemagglutination assay.

Hemagglutination assays were done in microtitre agglutination assay plate using MDA-MB-231 media in presence of serially diluted pectic polysaccharide in 0.15 M NaCl. The reaction mixture contained 150 µL of 4% erythrocyte suspension with or without serially diluted pectic polysaccharides. Minimum Inhibitory Concentration (MIC) of the substances was determined.

Determination of purity of active SRPP fraction – 0.15 M by HPLC

Of the two SRPP fractions, 0.15 M fraction showed higher activity. In order to ascertain the purity 10 mg of this was dissolved in deionized water (1 mL) and loaded on to E-linear and E-1000 column (4.0 x 250 cm) connected to a Shimadzu LC-10A.

HPLC system (Shimadzu Corp. Kyoto. Japan) Elution was performed with 100% Milli Q water as a mobile phase at room temperature at a flow rate of 0.8 mL/min and using RI detector. Emergence of peaks was monitored.

Determination of molecular weight of the active antimetastatic component

Gel permeation chromatography was performed on Sepharose CL-4B (1.6 cm x 92 cm). The active pectic polysaccharide fractions (10 mg) were dissolved in distilled water, centrifuged at 6000 *g* for 10 min at RT and the supernatant was loaded (1 mL) on to the column. The elution was carried out by 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. Dextran series standards (T-40, T-70, T-150, T-500, T-2000) were used to determine molecular weight. Blue dextran was used to determine void volume. A calibration curve was prepared by plotting V_e/V_0 versus log molecular weight. Where, V_e

= void volume, V_0 = elution volume. Molecular weight of the unknown polysaccharide was determined from this graph.

NMR spectral analysis of 0.15 M fraction

The active fraction of SRPP - 0.15 M fraction was dissolved in 1 mL of D₂O. After ensuring complete dissolution, the spectra were recorded with a Bruker amx 400 spectrometer at 500/700 MHz. IR spectral studies were performed to understand the extent of carboxymethyl reduction.

RESULTS

Extraction of pectic polysaccharide from Black cumin and Swallow root

Pectic polysaccharides were isolated from black cumin and swallow root using ammonium oxalate extraction method. Swallow root had the higher yield of pectic polysaccharide (6.2%), as it was a fleshy tuberous root, while the yield of pectic polysaccharide was very low in case of black cumin (0.8%) which had high amount of oils stored in it.

Antimetastatic property of pectic polysaccharides of black cumin (BCPP) and swallow root (SRPP)

Since our studies in the laboratory and results revealed the role of galectin-3 in inhibiting metastasis by enabling cancer cell to interact with normal cells via galectin-3 of cancer cell and beta-galactosides of extra cellular matrix of normal cells, galectin-3 inhibitory components were therefore considered as antimetastatic compounds. Antimetastatic activity thus was studied in different sources by galectin-3 interaction with red blood cells

which resulted in agglutination. Agglutination inhibition therefore was measured to understand the antimetastatic potency of the selected polysaccharide. In order to understand the relative efficiency of the sources in inhibiting galectin-3 mediated agglutination, minimum concentration required to inhibit agglutination was studied by serial dilution technique in the agglutination plate. A source which inhibits at lowest concentration was considered as the most potent source, MIC was therefore determined.

Pectic polysaccharides isolated from different dietary sources were evaluated for their galectin inhibitory activity based on hemagglutination assay. Minimum Inhibitory Concentration of the polysaccharide (MIC) in inhibiting the galectin mediated agglutination of red blood cells was determined and results were compared with standard galectin specific sugars - galactose and lactose. Results presented in Table 3.1 revealed that SRPP showed a potent agglutination inhibition with MIC of 1.85 $\mu\text{g/mL}$. MIC of 27.1 and 4.16 $\mu\text{g/mL}$ was observed for galactose and lactose that are specific for galectin-3 respectively. Citrus pectin had higher inhibition with MIC of 25 $\mu\text{g/mL}$ than, BCPP (130 $\mu\text{g/mL}$). Among the standards, lactose showed a good inhibitory activity with MIC of 4.16 $\mu\text{g/mL}$ than galactose (MIC-27 $\mu\text{g/mL}$). Data thus suggested that at least 15 and 2 fold increase in the activity was exhibited by SRPP over galactose and lactose respectively. Also, SRPP gave ~14 fold increased activity when compared to that of another reported source - citrus pectin which showed an MIC of 25 $\mu\text{g/mL}$.

Table 1: Agglutination inhibitory activity of various dietary polysaccharides, SRPP fractions. Lactose and Galactose against MDA-MB-231-galectin-3 induced hemagglutination of human erythrocytes.

Samples	Agglutination inhibition (MIC in $\mu\text{g eq. carbohydrate/mL}$)
Galactose	27.10
Lactose	4.16
SRPP	1.85
CPP	25
BCPP	130

Purification of SRPP

Since SRPP showed potent activity it was further fractionated on DEAE cellulose ion exchange chromatography. Upon purification, SRPP was fractionated in to four fractions namely 0.05, 0.10, 0.15 and 0.20 M ammonium carbonate eluted fraction (Figure 1).

Initially, a neutral fraction was also collected by water elution. The percentage yield of 0.15 M fraction was higher (62%) followed by neutral (17%), 0.10 M (15%) and 0.05 M (4%) fractions. These fractions were examined for antimetastatic activity. Results presented in Table 1 suggest that neutral, 0.1 M and 0.2 M fractions did not show any activity. While, 0.05 M and 0.15 M fraction showed potent antimetastatic activity with an MIC of 0.025 and 0.004 $\mu\text{g/mL}$ as opposed to that of the

crude SRPP which showed an MIC of 1.86 $\mu\text{g/mL}$. Increase of $\sim 74 - 400$ times in the activity in 0.05 and 0.15 M fraction indicate that it is due to increase in specific activity probably due to the elimination of inactive components during purification. Approximately 15-fold higher yield and 6-fold higher activity was

observed in 0.15 M fraction when compared to that of 0.05 M fraction. Since 0.15M fraction showed the better activity than all other fractions, the purity of the fractions examined on HPLC (Figure 2) provides the HPLC profile with the homogeneous peak.

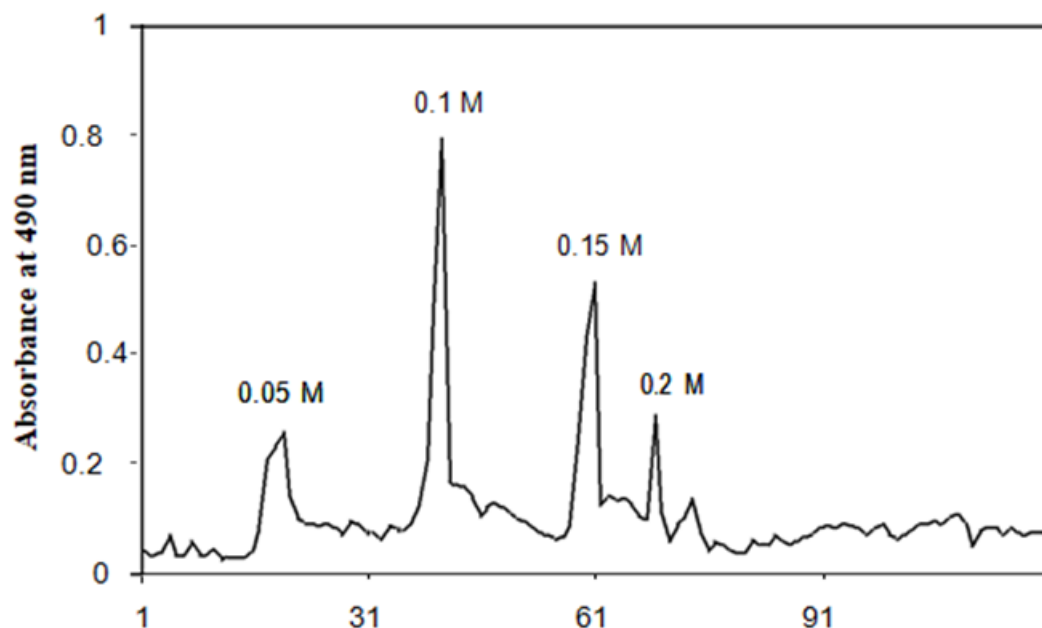


Fig. 1: DEAE cellulose column chromatography profile of SRPP.

Determination of purity of active SRPP 0.15 M fraction

The active SRPP fraction - 0.15 M fraction by HPLC was shown to be homogenous on gel permeation

chromatography on sepharose CL-4B column chromatography and HPLC analysis (Figure 2).

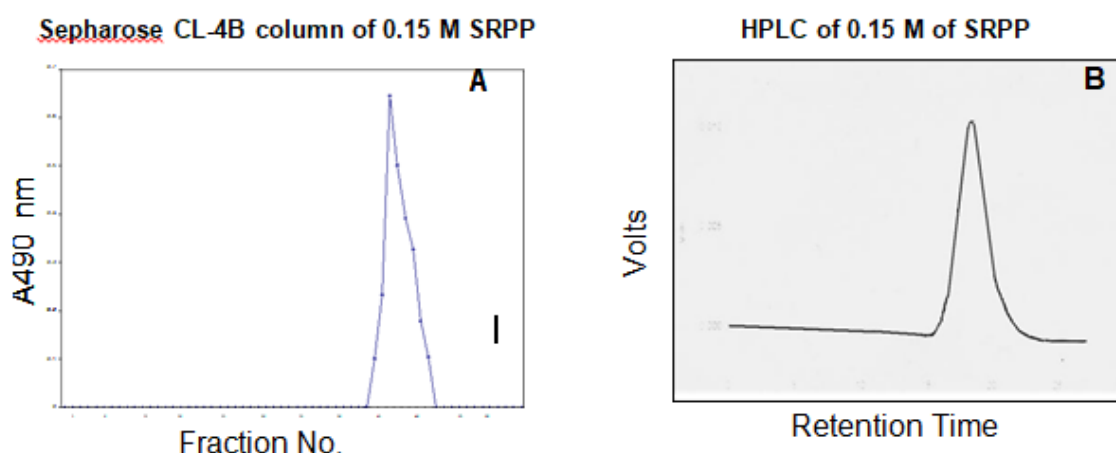


Fig. 2: Determination of purity of active SRPP fraction 0.15M by gel permeation chromatography on sepharose CL-4B column chromatography (A) and HPLC analysis (B).

Sugar composition analysis of SRPP and DEAE fractions of SRPP

Sugar composition analysis of galectin-3 inhibitory polysaccharide from swallow root, black cumin and citrus pectin were compared by GLC analysis. Data presented in Table 2 indicated the presence of rhamnose (16%), arabinose (50%), xylose (02%), galactose (32%)

in SRPP; rhamnose (05%), arabinose (30%), xylose (03%), galactose (19%), glucose (42%) in CPP and rhamnose (49%) arabinose (42%), xylose (02%), galactose (04%) and glucose (03%) in BCPP. In addition, uronic acid content of 141, 295 and 30 mg/g were observed for SRPP, CPP and BCPP, respectively suggesting the pectin nature.

As observed previously with different sources, differential activity was observed. Sugar composition analysis was determined in order to verify whether arabinose and galactose are present in these fractions. Swallow root pectic polysaccharide of 0.05 M and 0.15 M fractions which exhibited antimetastatic activity with an MIC of 0.025 µg/mL and 0.004 µg/mL did not contain significant content of rhamnose, mannose and glucose although 7% of rhamnose was found in 0.15M fraction, indicating that these sugars are not involved in the activity. Arabinose was absent in 0.05 M SRPP fraction suggesting that arabinose may not be crucial for the activity. Also 0.1 M and 0.2 M fractions which contained higher levels of arabinose 83 and 100%, respectively did not show activity supporting the fact that arabinose may not be a crucial sugar for the activity. Data thus points to the fact that galactose may be a crucial sugar component

responsible for the activity. Further, 0.05 M fraction contained ~ 2.7 fold higher levels of galactose (76%) as opposed to only 27% in 0.15 M fraction. If only galactose is important for the activity, 0.05 M should have showed better activity than 0.15 M fraction. Together data may suggest that both arabinose and galactose may be important for the activity. A definite proportion of arabinose and galactose hence may be important for the activity. However this alone do not explain 6-fold increase in activity in 0.15 M over 0.05 M, since ~ 37 fold difference in uronic acid content were observed in 0.15 M fraction. Uronic acid may also be important for the activity. Precise structure - function analysis is required to completely understand the requirement for pectic polysaccharide to be antimetastatic, in addition to the presence of galactose as a basic requirement.

Table 2: Relative percent sugar composition analysis and uronic acid contents of pectic polysaccharides from various sources. SRPP – swallow root pectic polysaccharide; CPP - citrus pectic polysaccharide and BCPP – black cumin pectic polysaccharide.

Sugars	SRPP	CPP	BCPP
Rhamnose	16	05	49
Arabinose	50	30	42
Xylose	02	03	02
Mannose	-	-	-
Galactose	32	19	04
Glucose	-	43	03
Uronic acid (mg/g)	141	295	30

Table 3: Relative percent sugar composition and uronic acid content of DEAE fractionated swallow root pectic polysaccharide.

Sugars	SRPP	0.05 M	0.10 M	0.15 M	0.20 M
Rhamnose	16	-	17	7	-
Arabinose	50	-	83	66	100
Xylose	02	24	-	-	-
Mannose	-	-	-	-	-
Galactose (Free Gal)	32	76	-	27	-
Glucose	-	-	-	-	-
Uronic acid (mg/g)	141	3.0	21	113	1.0

Determination of NMR analysis of active 0.15 M pectic polysaccharide fraction of SRPP

Although the inactive fractions do not also contain higher levels of uronic acids, there is no direct evidence for correlation between uronic acid content and the activity. The nature of uronic acids present in them may be responsible for the same. Hence detailed structural studies were undertaken for SRPP. The ^{13}C NMR spectral profile obtained for 0.15 M fraction of SRPP showed (Figure 3.4) the presence of characteristic pectic

type polysaccharide. The galacturonic acid main chain occurring as a back bone of the polysaccharide has been observed. The six signals at 100.41, 69.54, 70.42, 79.44, 72.86 and 176.76 ppm corresponds to the ring carbon C-1, C-2, C-3, C-4, C-5 & C-6 respectively of 1,4 α linked D-galactopyranosyluronic acid units. The signal at 62.54 is assigned to C-6 of β -Galactose residue linked to α – arabinose by 1,4 linkage. The signal at 22.23 is possibly due to the presence of methyl groups. This suggests that the pectic polysaccharide is methylated.

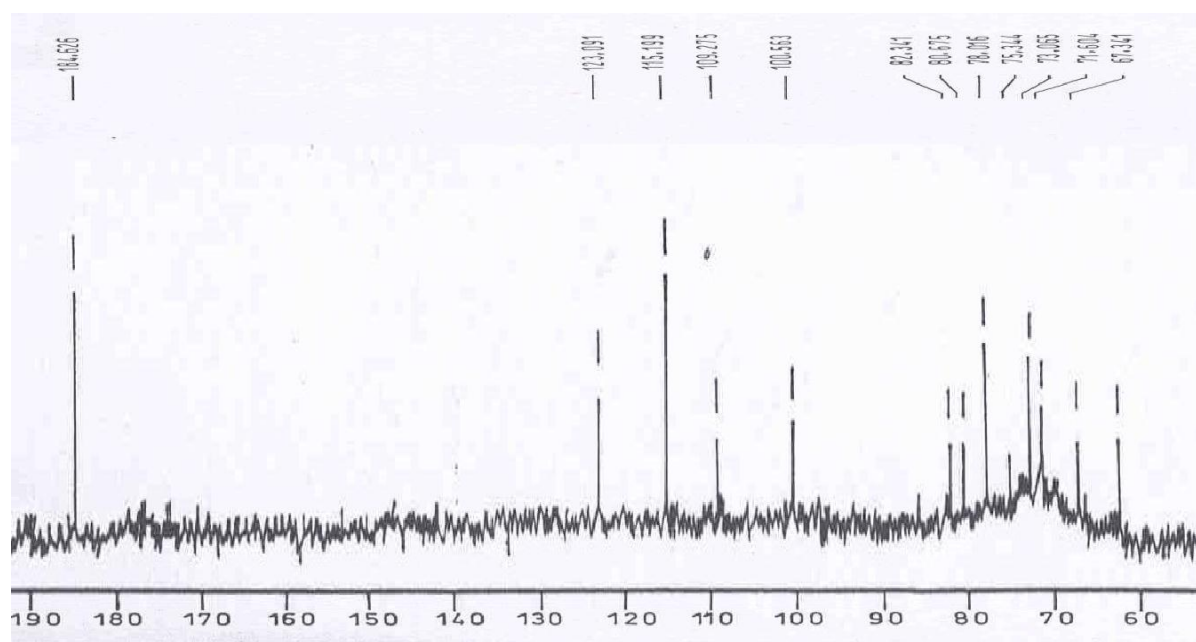


Figure 3: NMR analysis of active fraction of SRPP - 0.15 M fraction.

DISCUSSION

In the present study, we isolated pectic polysaccharides from swallow root and black cumin to examine their ability to block galectin-3 molecule reported to be involved in metastasis progression of various types of cancers (Song *et al.*, 2002). Galectin binding activity of pectic polysaccharide is of importance because galectin is a key molecule in metastasis which functions by binding to $\square$ -galactosides on the normal cell. Ability of pectic polysaccharide to bind to galectin may inhibit or hinder the interaction of galectin from cancer cell to normal cells which triggers metastasis.

Galectin-3 inhibitory activity of pectic polysaccharides isolated from black cumin and swallow root was examined in agglutination assay to evaluate galectin-3 binding activity. Among the sources studied swallow root yielded higher amount of pectic polysaccharide. Also, swallow root pectic polysaccharide showed comparatively higher (70-fold) galectin-3 inhibitory activity than black cumin pectic polysaccharide. Hence, SRPP was taken for further characterization.

Results of the study indicated the association between arabinose and galactose with activity. Pectic polysaccharide from different sources showed different

activity and also differences in sugar composition. In order to understand the relation between type of sugars in selected pectic polysaccharides and antimetastatic activity, correlation coefficient-R was determined. Results indicated a correlation coefficient of 0.06851, 0.6341, -0.1678, 0.695 and -0.3922 for rhamnose, arabinose, xylose, galactose and glucose respectively. A strong correlation was observed between arabinose/galactose and antimetastatic activity (higher arabinose/galactose - higher antimetastatic activity). Significant inverse relationship between rhamnose and antimetastatic activity was also observed (higher rhamnose content – low activity); while no significant correlation existed between the activity and other sugars. Results thus suggest that the presence of galactose and arabinose appear to be crucial for the activity of the sources studied. Further, ~6.25-fold higher active SRPP fraction (0.15 M) contained 2.8-fold lesser galactose than that found in 0.05 M fraction, but contained additionally 66% of arabinose indicating that both arabinose and galactose may be important for the activity. Further, to confirm the fact that arabinose and galactose are important for the galectin-inhibitory property, purified larch wood arabinogalactan that contained 98% (w/w) of arabinogalactan was examined for galectin inhibitory property. Galectin inhibitory activity with an MIC of

~200 µg/mL was obtained, indicating that not only the presence of arabinose and galactose may be essential for the activity; while a precise arabinose-galactose structure may be involved.

Current study has a greater implication in that it is supporting a workable concept of probable prevention of metastasis. This is the subsequent 2nd report apart from citrus pectin and 1st report in spices with antimetastatic potency. Nevertheless as indicated in results section, SRPP had higher potency since it showed ~20-fold higher activity than citrus pectin. Besides this SRPP also contain bound phenolics (Srikanta *et al.*, 2007) which may also contribute significantly to anticancer property.

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