Chapter I.3

Sulphated galactans of *Sarconema filiforme* and *Sarconema scinaioides*

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3 Sulphated galactans of *Sarconema filiforme* and *Sarconema scinaioides*

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I.3.1 INTRODUCTION

Sulphated galactans, agars and carrageenans are the main cell wall components of the red seaweeds, are composed of repeating dimeric unit of (1→3)-linked β-galactopyranose (Galp) and (1→4)-linked 3,6-anhydro α-Galp (Craigie 1990). The (1→4)-linked galactose units have L and D configuration in agar and carrageenans respectively (Rees 1969). The number and position of sulphated ester (S), (1→3)-linked β-galactopyranose (G unit) and the occurrence of 3,6-anhydro bridges in the (1→4)-linked residues (DA unit) determines the type of the carrageenan unit (Knutsen et al. 1994). The presence of one, two and three ester-sulphate groups per repeating disaccharide unit are the characteristics feature of three main carrageenans viz. kappa- (κ, DA-G4S), iota- (ι, DA2S-G4S), and lambda- (λ, D2S6S-G2S) respectively (Knutsen et al. 1994). Besides these sulphated esters and 3,6-anhydro bridges, red algal galactans may also bear pyruvate acetal substitutions as well as glycosyl substitutions, most commonly known as mono-O-methyl galactopyranosyl (Me-Galp) residues branching out from the main galactan chain (Painter 1983; Falshaw et al. 1996; Chiovitti et al. 1997; Usov 1998). The extraction procedures of the polysaccharides, seaweeds sources and life stages, influence the structural complexity and hybrid nature of the carrageenans (Craigie 1990; Knutsen et al. 1994; Bixler 1996; Van de Velde et al. 2001). The presence of biosynthetic precursor e.g. mu- (µ, D6S-G4S, κ-carrageenan precursor) and nu (ν, D2S6S-G4S, ι carrageenan precursor) also hinders the gellation of the respective carrageenans (Bellion et al. 1983; Van de Velde et al. 2002). Varieties of industrial and laboratory applications (e.g. stabilising and viscosity building agent etc.) are accounted for these carrageenans due to their viscous nature as well as gelling properties (Villanueva et al. 2004).

κ-Carrageenan forms hard gels with KCl solution which are strong and brittle, whereas ι-carrageenan forms soft and weak gels that are shear reversible (Villanueva et al. 2004, Campo et al. 2009). Recently hybrid carrageenans have attracted attention of the researchers world over because of its potential in industrial applications especially in the food and personal care industries (Piculell 1995). Several red seaweeds have been explored for their κ/ι-hybrid carrageenans contents (Chiovitti et al. 2001).

\textit{Sarconema filiforme} (Sonder Kylin) and \textit{Sarconema scinaioiides} Børgesen are red seaweed species abundantly available in Indian waters. These seaweeds belong to Division-Rhodophyta (Class- Rhodophyceae, order-Gigartinales, family- Solieriaceae, genus-\textit{Sarconema} and species- filiforme and scinaioiides; Jha et al. 2009). These
seaweed species occur at the Indian coast along with other various marine algal species indicating that these species are well adopted in their habitat.

Carrageenans extracted from Sarconema filiforme have been reported earlier (Semesi and Mshigeni 1977; Parekh et al. 1988; Chiovitti et al. 1998; Rajasulochana and Gunasekaran 2009). ι-Carrageenan has been reported from Sarconema filiforme of Tanzanian as well as from Indian waters (Semesi and Mshigeni 1977; Parekh et al. 1988; Rajasulochana and Gunasekaran 2009), while a hybrid of α/ι carrageenans and pyruvated α carrageenan was reported from the Australian waters (Chiovitti et al. 1998). The aim of this study is detailed chemical characterization of the carrageenan of Sarconema filiforme of Indian waters and comparison of the data with those of the same seaweed species reported only by IR data (Parekh et al. 1988; Rajasulochana and Gunasekaran 2009).

Carrageenans extracted from Sarconema scinaioides have been earlier reported as hybrid of κ/ι carrageenans containing pyruvated moieties on the basis of 1H and 13C NMR data (Van de Velde et al. 2005). The aim of this study is the characterization of the carrageenan extracted from Sarconema scinaioides of Indian waters in an ongoing program on value addition of seaweeds.

I.3.2 MATERIALS AND METHODS

I.3.2.1 Collection of seaweeds

Sarconema filiforme and Sarconema scinaioides used in the present study were collected in March 2008 from Okha (22° 28´ N, 69° 04´ E) and in January 2007 from Veraval (20° 55´ N, 70° 20´ E) respectively, from the inter-tidal zone in the west coast of India (Oza and Zaidi, 2001; www.algaebase.org). The seaweed thalli were washed with sea water to remove impurities and air dried. The voucher specimens of Sarconema filiforme (AL-II-126-09) and Sarconema scinaioides (AL-II-104-03) were deposited with the herbarium of the CSMCRI, Bhavnagar for references. Borane 4-methyl morpholine complex (MMB) and α-amylase (from Bacillus amyloliquefaciens, Enzyme Code. 3.2.1.1) were purchased from Sigma-Aldrich. Other chemicals used in this study were AR grade except for isopropyl alcohol (LR grade), which were purchased from M/s S D Fine Chemicals, Mumbai.

I.3.2.2 Extraction of sulphated galactans

Dried seaweed (50 g) was soaked in demineralized (DM) water for 1h followed by extraction with 0.05 M NaHCO3 (1:30 w/v) at 110 °C for 2h in an autoclave (Craigie and Leigh,1978). The cooked seaweed was then homogenized;
centrifuged and crude sulphated polysaccharide was isolated from the supernatant by precipitation with isopropyl alcohol (IPA; 1:2 v/v), followed by washing with aqueous isopropyl alcohol (90 & 95% v/v), and finally by pure isopropyl alcohol to yield SF\textsubscript{Crude} and SS\textsubscript{Native} respectively. It may be noted that the galactan of \textit{Sarconema scinaoides} (SS\textsubscript{Native}) contains floridean starch which was removed by treatment with $\alpha$-amylase to get floridean starch digested (detected by iodine test, GC-MS and $^{13}$C NMR), reheated, centrifuged followed by dialysis and precipitated with isopropyl alcohol (1:2 v/v), followed by washing with aqueous isopropyl alcohol (90 & 95% v/v), and finally by pure isopropyl alcohol to get SS\textsubscript{Crude} (Falshaw and Furneaux, 1998; Oza \textit{et al.} 2011).

I.3.2.3 Alkaline modification and desulphation of sulphated galactans

SF\textsubscript{Crude} and SS\textsubscript{Crude} were alkali modified using NaBH$_4$/NaOH system to give SF\textsubscript{AM} and SS\textsubscript{AM} respectively (Falshaw and Furneaux 1998; Viana \textit{et al.} 2004). Briefly, samples (1 gm) were dissolved in NaOH solution (3M, 100 ml) and NaBH$_4$ (1 gm) was added to it. The contents were stirred overnight and then 200 ml of water was added to lower the concentration of NaOH in the reaction mixture from 3M to 1M. Additional NaBH$_4$ (1 gm) was added and the contents were heated at 80 °C for 4 h. After completion of the reaction the contents were diluted to 1 litre with water and cooled to room temperature. The solution was neutralised with acetic acid solution (10 % w/v) and then dialysed followed by freeze drying.

Solvolytic desulphation of pyridinium salts of SF\textsubscript{AM} and SS\textsubscript{AM} were done according to the method described by Falshaw and Furneaux (1998). The polysaccharide solution (0.1 % w/v) was dialyzed against pyridine hydrochloride solution (1000 ml, 0.1 M) for overnight followed by dialyses against water (1000 ml x 4). The dialyzate were then freeze dried to get pyridinium salts of polysaccharides. The pyridinium salts (200 mg) of each samples were dissolved in 100 ml of a mixture of anhydrous DMSO-MeOH-Pyridine (89:10:1 v/v) and heated at 100 °C for 4h. After completion of the reaction the contents were cooled to room temperature, distilled water (100 ml) was added and the mixture were dialyzed overnight against distilled water and freeze-dried to afford desulphated polysaccharide SF\textsubscript{Des} and SS\textsubscript{Des} respectively.

I.3.2.4 General Methods and analysis

Estimation of protein

Protein contents were calculated from the percentage nitrogen (%N) estimated by Kjeldahl’s method on a KEL PLUS- KES 201 Digestion unit attached to a KEL.
PLUS-CLASSIC DX Distillation unit (M/s PELICAN equipments, Chennai, India) multiplying the %N value with the conversion factor 6.25 (Marks et al. 1985).

Estimation of total sugar

Sugars were estimated using the method described by Dubois et al. (1956) as given in section I.2.2.5.

Estimation of pyruvate content

The pyruvate contents of samples were determined using the 2,4-dinitrophenylhydrazine (DNPH) method as described by Sloneker and Orentas (1962). Sodium pyruvate was used for preparation of the calibration curve. Sodium pyruvate (1-5 mg) as references and samples (10 mg) were hydrolyzed with 10 ml of 3.5 N HCl at 110 °C overnight. To the 2 ml of the hydrolysate, DNPH solution (1 ml, 0.5 % w/v in 2N HCl) was added and the reaction mixture was kept at room temperature for 5 min. Ethyl acetate 10 ml was added and the content was well on a vortex mixer. The lower aqueous layer was discarded. The ethyl acetate layer was washed with aq. Na₂CO₃ solution (10 ml x 2; 10% w/v). The lower aqueous Na₂CO₃ layers were combined and OD was recorded at 375 nm. The calibration curve was prepared using sodium pyruvate as references in the range of 0.2 to 1 mg of sodium pyruvate. Each analysis were done in triplicate and mean value were considered.

Specific rotation

Optical rotations were measured for SFCrude and SSCrude (0.25% w/v; at 30 °C used wavelength 589 nm) on a Rudolph Digipol-781 Polarimeter (Rudolph Instruments Inc, NJ, USA).

Viscosity

Apparent viscosity of SFCrude, SSCrude and t-carrageenan (1% w/v, in DM water, in 1% salt solutions) were measured using a Brookfield Viscometer (DV-II+Pro) at 80 °C using spindle SC4-18 for SFCrude and LV3 for SSCrude and t-carrageenan at speed of 30 rpm.

Metal and sulphate content analysis

Metal ion analyses were carried out after ignition of a known weight of SFCrude and SSCrude followed by acid digestion. Volume of digested samples was adjusted to
100 ml with distilled water. Metal ions (Ca, Cd, Co, Cr, Cu, Fe, K, Mg, Na, Ni, Pb, As, B, Zn) as well as sulphur contents were measured by inductively coupled plasma (ICP) spectrophotometry on a Perkin-Elmer ICP-OES Optima 2000DV machine, following the method described by Wolnik (1988). Only sulphur contents of SF_{AM}, SF_{Des}, SS_{Native}, SS_{AM} and SS_{Des} were determined as described above.

**Rheological analysis**

The flow behavior of the SF_{Crude} (2.5 % w/v in water and in 1% salt solutions), as well as of SS_{Crude} and iota carrageenan (1.0 % w/v in 1% salt solutions) were studied on an Anton-Paar Physica MCR 301 Rheometer, Germany, employing plate/plate geometry (50 mm diameter) at 25 °C applying 0-1000 s^{-1} shear rate.

**Molecular weight determination (GPC)**

Same method has been followed as described in section (I.2.2.5) for the determination of molecular weights (Mn, Mw, Mz and Mp) as well as polydispersity of SF_{Crude}, SF_{AM}, SF_{Des}, SS_{Crude}, SS_{AM} and SS_{Des}. The molecular weights of standards and samples were determined by GPC according to the method described in literature (Yamamoto *et al.* 1995; Li *et al.* 2008).

**I.3.2.5 Spectroscopic analysis**

**Infrared spectroscopy**

FTIR spectra of SF_{Crude}, SF_{AM}, SF_{Des}, SS_{Native}, SS_{Crude}, SS_{AM} and SS_{Des} were recorded on a Perkin-Elmer Spectrum GX (FT-IR System, USA), using the KBr disk method by taking ~5.0 mg of sample in ~600 mg KBr. All spectra were average of two counts with 10 scans each and a resolution of 5 cm^{-1}.

**^{13}C NMR spectroscopy**

^{13}C NMR spectra of SF_{Crude}, SS_{Native}, SS_{Crude} and SS_{AM} were recorded on a Bruker Avance-II 500 (Ultra shield) Spectrometer, Switzerland, at 125 MHz. Sample was dissolved in D_{2}O (50 mg/mL) and the spectra was recorded at 70 °C, using DMSO as internal standard (ca. δ 39.4 ppm).
$^1$H, 2D $^1$H, $^1$H COSY and $^1$H/$^{13}$C HSQC NMR of SF$_{Crude}$ and SS$_{AM}$

$^1$H NMR as well as 2D $^1$H/$^1$H COSY (Homonuclear correlation spectroscopy) and $^{13}$C/$^1$H HSQC (Heteronuclear Single Quantum Coherence) correlation spectrum analysis of SF$_{Crude}$ and SS$_{AM}$ were recorded on a Bruker Avance-II 500 (Ultra shield) spectrometer, Switzerland, at 500 and 125 MHz (for $^1$H and $^{13}$C respectively) at 70 °C. Default programs of Bruker were used for COSY and HSQC spectra using DMSO as the internal signal (ca. 2.68 ppm for $^1$H and 39.43 ppm for $^{13}$C).

I.3.2.6 Monosaccharides composition and linkages analysis

Preparation of alditol acetate of polysaccharide by reductive hydrolysis and GC-MS analysis

Monosaccharide composition in SF$_{Crude}$, SF$_{AM}$, SF$_{Des}$, SS$_{Native}$, SS$_{Crude}$, SS$_{AM}$ and SS$_{Des}$ were determined quantitatively as their peracetylated alditols obtained by reductive hydrolysis followed by acetylation as described by Stevenson and Furneaux (1991). The analysis of resulting alditol acetates were carried out on a Shimadzu GC-MS-QP2010 machine, using a SGE BP-225 capillary column (25m, 0.25µm, 0.22mm), employing temperature programming 160°C (3 min hold) to 230 °C (10°C/min), split ratio 1:30 and a flame ionization detector (FID) as given in the section I.2.2.7 (Siddhanta et al. 2001).

Methylation of SF$_{Crude}$, SF$_{AM}$, SF$_{Des}$, SS$_{Native}$, SS$_{AM}$ and SS$_{Des}$

Permethylation of SF$_{Crude}$, SF$_{AM}$, SF$_{Des}$, SS$_{Native}$, SS$_{AM}$ and SS$_{Des}$ were done as described by Ciucanu and Kerek (1984). Permethylated alditol acetates (PMAAs) were obtained by reductive hydrolysis and acetylation (Stevenson and Furneaux 1991). The sample (100 mg) was suspended in a round bottom flask (fitted with a rubber septum) containing 25 ml of dry dimethyl sulfoxide (DMSO) and ~1.0 g powdered NaOH was stirred at 5 to 10 °C, to which methyl iodide (CH$_3$I) was added in three parts (1 ml x 3) at an interval of 30 min. The reaction mixture was stirred overnight. Nitrogen gas stream was then purged into the reaction mixture carefully to remove unreacted methyl iodide. The reaction mixture was then dialyzed against tap water followed by DM water to remove DMSO and unreacted reagents. After dialysis, the product was freeze dried. Partially methylated alditol acetates (PMAAs) were obtained by reductive hydrolysis and acetylation from the methylated samples using the method described above.
Linkage analysis of SF\textsubscript{Crude}, SF\textsubscript{AM}, SF\textsubscript{Des}, SS\textsubscript{Native}, SS\textsubscript{AM} and SS\textsubscript{Des}

GC-MS analysis of the partially methylated alditol acetate of SF\textsubscript{Crude}, SF\textsubscript{AM}, SF\textsubscript{Des}, SS\textsubscript{Native}, SS\textsubscript{AM} and SS\textsubscript{Des} were carried out on a Shimadzu GC-MS-QP2010 machine, using a SGE BP-225 capillary column (25m, 0.25µm, 0.22mm), employing temperature programming 160°C (3 min hold) to 230 °C (10°C/min), split ratio 1:30 and a flame ionization detector (FID) as mentioned above (Siddhanta \textit{et al.} 2001). The electron impact (EI) mass-spectra were recorded at 70 eV. The mass fragmentation patterns of the PMAAs of SF\textsubscript{Crude}, SF\textsubscript{AM}, SF\textsubscript{Des}, SS\textsubscript{Native}, SS\textsubscript{AM} and SS\textsubscript{Des} were compared and validated with those of PMAAs reported by Sassaki \textit{et al.} (2005) as well as with those provided by CCRC data bank (http://www.ccrc.uga.edu/specdb/ms/pmaa/pframe.html). The sugar residues in the sample were identified by comparing their unique mass fragmentation patterns (http://www.ccrc.uga.edu/specdb/ms/pmaa/pframe.html).

\section*{I.3.3 RESULTS AND DISCUSSION}

Samples denoted with SF and SS were generated from \textit{Sarconema filiforme} and \textit{Sarconema scinaiioides} respectively.

\subsection*{I.3.3.1 Physicochemical results}

The yields of the SF\textsubscript{Crude}, SF\textsubscript{AM} and SF\textsubscript{Des} were 31.0, 23.1 and 14.0 % while those of SS\textsubscript{Native}, SS\textsubscript{Crude}, SS\textsubscript{AM} and SS\textsubscript{Des} were 31.0, 27.8, 24.2 and 16.8 % with respect to as received dry seaweed respectively (Table I.3.1). Total sugar, protein and sulphate content as well as monosaccharides composition analysis of SF\textsubscript{Crude}, SF\textsubscript{AM}, SF\textsubscript{Des}, SS\textsubscript{Native}, SS\textsubscript{Crude}, SS\textsubscript{AM} and SS\textsubscript{Des} have been given in Table I.3.1. The sulphate content (SO\textsubscript{4}^2-) in SF\textsubscript{Crude} was 25.1 % w/w, as opposed those reported earlier from the same seaweed species from Indian, Tanzanian and Australian waters describing 21.3%, 18.3% and 25.0% w/w sulphate respectively (Semesi and Mshigeni 1977; Parekh \textit{et al.} 1988; Chiovitti \textit{et al.} 1998). The alkaline modification of SF\textsubscript{Crude} reduces sulphate content by 1.9 % in SF\textsubscript{AM}. The pyruvate content in SF\textsubscript{Crude} and SF\textsubscript{AM} were found to be 3.6 and 4.3 % respectively.

The yields of the native, crude, alkali modified and desulphated carrageenan of \textit{Sarconema scinaiioides} were 31.0 %, 27.8 %, 24.2 % and 16.0 % with respect to dry seaweed, respectively. The \(\alpha\)-amylase treatment of SS\textsubscript{Native} decreased the yield to 27.8 % in SS\textsubscript{Crude} which may be due to digestion of floridean starch. Alkali modification of SS\textsubscript{Crude} resulted in increase (ca. 2.1%) in sulphate content presumably
because of leach out of polysaccharides during alkaline modification. The total sugar and sulphated content of SS\textsubscript{Native}, SS\textsubscript{Crude}, SS\textsubscript{AM} and SS\textsubscript{Des} are shown in the Table I.3.1.

**Table I.3.1:** Physicochemical and monosaccharides analysis of sulphated galactans of *Sarconema filiforme* and *Sarconema scinaioides*

<table>
<thead>
<tr>
<th>Extract</th>
<th>Yield\textsuperscript{a}</th>
<th>Total Sugar\textsuperscript{b}</th>
<th>Protein\textsuperscript{b}</th>
<th>Sulphate\textsuperscript{b}</th>
<th>Xyl 3,6-&lt;br&gt;AnGal</th>
<th>6-O-Me Gal</th>
<th>Gal</th>
<th>Glc</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF\textsubscript{Crude}</td>
<td>31.0</td>
<td>45.4</td>
<td>3.2</td>
<td>25.1</td>
<td>-</td>
<td>23.0</td>
<td>9.8</td>
<td>64.5</td>
</tr>
<tr>
<td>SF\textsubscript{AM}</td>
<td>23.1</td>
<td>55.3</td>
<td>2.7</td>
<td>23.2</td>
<td>-</td>
<td>29.8</td>
<td>7.6</td>
<td>59.6</td>
</tr>
<tr>
<td>SF\textsubscript{Des}</td>
<td>14.0</td>
<td>60.2</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>19.3</td>
<td>10.2</td>
<td>68.4</td>
</tr>
<tr>
<td>SS\textsubscript{Native}</td>
<td>31.0</td>
<td>45.2</td>
<td>ND</td>
<td>25.2</td>
<td>2.3</td>
<td>18.9</td>
<td>15.1</td>
<td>41.2</td>
</tr>
<tr>
<td>SS\textsubscript{Crude}</td>
<td>27.8</td>
<td>46.1</td>
<td>ND</td>
<td>26.2</td>
<td>3.2</td>
<td>24.4</td>
<td>12.7</td>
<td>59.7</td>
</tr>
<tr>
<td>SS\textsubscript{AM}</td>
<td>24.2</td>
<td>47.2</td>
<td>ND</td>
<td>28.3</td>
<td>-</td>
<td>28.4</td>
<td>9.3</td>
<td>62.3</td>
</tr>
<tr>
<td>SS\textsubscript{Des}</td>
<td>16.8</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
<td>-</td>
<td>22.2</td>
<td>10.8</td>
<td>67.0</td>
</tr>
</tbody>
</table>

\textsuperscript{a}Yield (in % w/w) was calculated on the basis of as received dry seaweeds and are mean values of the three replicates, \textsuperscript{b}Yield was w.r.to galactan polysaccharides (in % w/w), ND: Not determined; '-' : Absent

**Optical rotation, apparent viscosity and metal content analysis**

The optical rotation and apparent viscosity data of SF\textsubscript{Crude}, SS\textsubscript{Crude} and \(\tau\)-carrageenan are given in the Table I.3.2. The SF\textsubscript{Crude} SS\textsubscript{Crude} and \(\tau\)-carrageenan exhibited positive specific rotations \([\alpha]_D^{30} +17.23^{\circ}, + 31.19^{\circ} and + 39.72^{\circ}\) respectively (c 0.25%, \(\text{H}_2\text{O}\), 30 °C) indicating that these galactans belonged to carrageenan family. The apparent viscosities of SF\textsubscript{Crude} were of the following order in: water < KCl < CaCl\(_2\) < KCl & CaCl\(_2\) (1:1) indicating the existence of a stronger gel network formed due to the cross linking with salts. It further validated the fact that the SF\textsubscript{Crude} contained \(\tau\)-carrageenan, showing greater increase in viscosity in presence of Ca\(^{2+}\) ions compared to K\(^+\) ions (Craigie and Leigh, 1978). Apparent viscosities of SS\textsubscript{Crude} and \(\tau\)-carrageenan showed the following order in: water < KCl < KCl & CaCl\(_2\) (1:1) < CaCl\(_2\) indicating the existence of a stronger gel network formed due to the cross linking with CaCl\(_2\). It validated the fact that the carrageenan was of \(\tau\)-variety, showing greater increase in viscosity in the presence of Ca\(^{2+}\) ions compared to K\(^+\) ions (Morris & Belton, 1982). High viscosity of SF\textsubscript{Crude} and SS\textsubscript{Crude} in the presence of salts makes it potentially useful for various applications.

Metal ion contents of SF\textsubscript{Crude} and SS\textsubscript{Crude} were measured by inductively coupled plasma (ICP) spectrophotometry and the results are presented in Table I.3.2. The absence/negligible content of prominent toxic metal ions e.g. Cd, Pb, Cr and As in these sulphated galactans suggested that these sulphated galactans would be suitable for ingestible applications. The result of metal content analysis suggests that
K, Ca and Na were main ions attached with sulphated group of the carrageenan skeleton as expected.

Table I.3.2 Optical rotation, apparent viscosity and metal content analysis of SF\textsubscript{Crude} SS\textsubscript{Crude} and \textit{t-}carrageenan

<table>
<thead>
<tr>
<th>Extract</th>
<th>Optical rotation ([\alpha]_D^{30})</th>
<th>Apparent viscosity (in cP)(^a)</th>
<th>Metal ions(in ppm)(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Water</td>
<td>KCl</td>
<td>CaCl(_2) KCl &amp; CaCl(_2) (1:1)</td>
</tr>
<tr>
<td>SF\textsubscript{Crude}</td>
<td>17.23°</td>
<td>10</td>
<td>15 24 68</td>
</tr>
<tr>
<td></td>
<td></td>
<td>K (40200), Ca (33600), Cr (Nil), Fe (1.3), Co (Nil), Mn (1.9), Ni (0.3), Zn (4.3), Cu (2.4), Cd (Nil), As (Nil), Pb (Nil)</td>
<td></td>
</tr>
<tr>
<td>SS\textsubscript{Crude}</td>
<td>31.19°</td>
<td>7</td>
<td>15 45 32</td>
</tr>
<tr>
<td></td>
<td></td>
<td>B (6.1), Na (16200), Mg (26.5), K (49000), Ca (35000), Cr (Nil), Fe (0.9), Co (Nil), Mn (0.8), Ni (0.4), Zn (3.5), Cu (2.1), Cd (Nil), As (Nil), Pb (Nil)</td>
<td></td>
</tr>
<tr>
<td>\textit{t-}carrageenan</td>
<td>39.72°</td>
<td>6</td>
<td>13 39 28 ND</td>
</tr>
</tbody>
</table>

\(^a\) Apparent viscosities were measured of 1% (w/v) in water and 1% salt solutions at 80°C, \(^b\) w.r.to polysaccharide w/w, ND-Not determined

Rheological analysis

Flow behavior of solution of SF\textsubscript{Crude} (1% w/w) in presence of salts exhibited reduction of shear viscosity with shear rate in the following order, water < KCl < CaCl\(_2\) < KCl & CaCl\(_2\) (1:1) indicating existence of a stronger gel network formed due to the cross linking with salts (Figure I.3.1). Similar result was obtained in the flow behavior of SS\textsubscript{Crude} and iota carrageenan. Both of them exhibited reduction of shear viscosity with shear rate in the following order, KCl < CaCl\(_2\) indicating existence of a stronger gel network formed due to the cross linking with CaCl\(_2\) (Figure I.3.2). These values were in accordance with the apparent viscosity data (Table I.3.2).

Molecular weight determination (GPC)

The gel permeable chromatograms of SF\textsubscript{Crude}, SF\textsubscript{AM}, SF\textsubscript{Des}, SS\textsubscript{Native}, SS\textsubscript{Crude}, SS\textsubscript{AM} and SS\textsubscript{Des} are shown in the Figure I.3.2. The molecular weights (M\(_n\), M\(_w\), M\(_p\) and M\(_z\)) and polydispersity are given in the Table I.3.3. The high polydispersity indices indicated branched, non-homogeneous structure for these polysaccharides unlike synthetic polymers.
I.3.3.2 Spectral analyses

FT-IR spectroscopy

The appearance of the strong IR band in SF\textsubscript{Crude}, SF\textsubscript{AM}, SF\textsubscript{Des}, SS\textsubscript{Native}, SS\textsubscript{Crude}, SS\textsubscript{AM} and SS\textsubscript{Des} at 1255 cm\textsuperscript{-1} indicated the presence of sulphated ester, the bands at 931, 803, 846 cm\textsuperscript{-1} (in SF\textsubscript{Crude}, SF\textsubscript{AM} and SF\textsubscript{Des}) and at 931, 806, 853 cm\textsuperscript{-1} (in SS\textsubscript{Native}, SS\textsubscript{Crude}, SS\textsubscript{AM} and SS\textsubscript{Des}) confirmed the presence of 3,6-AnGal, axial sulphate ester at O-2 of 4-linked 3,6 AnGal and sulphate ester at O-4 of 3-linked Gal residues, respectively (Figure I.3.4 and I.3.5; Matsuhiro and Rivas 1993; Pereira \textit{et al}. 2003; Yermak \textit{et al}. 2006). Other characteristic bands at 1637, 1156, 1079, 1024 cm\textsuperscript{-1} were due to –O-H bending, C-O-C stretching of 6-O-methylated Gal, C-O stretching of secondary and primary alcohols of pyranose ring respectively (Matsuhiro and Rivas 1993; Kacuráková \textit{et al}. 2000; Pereira \textit{et al}. 2003). The presence of 3-linked Gal residues bearing pyruvate acetal substitution was confirmed by the appearance of the band at 897 cm\textsuperscript{-1} in galactans of \textit{Sarconema filiforme} (Chiovitti \textit{et al}.1997). Thus this leads one to conclude that SF\textsubscript{Crude} and SF\textsubscript{AM} were actually a hybrid of \(\alpha\) and \(\iota\) carrageenans having pyruvated acetal substitution (Stevenson and Furneaux 1991; Chiovitti \textit{et al}.1997). The absence of sulphate ester IR bands in SF\textsubscript{Des} 1255, 803 and 846 cm\textsuperscript{-1} at and in SS\textsubscript{Des} at 1255, 806 and 853 cm\textsuperscript{-1} confirmed the fact that they were the completely desulphated products of SF\textsubscript{AM} and SS\textsubscript{AM} respectively (Figure I.3.4c and I.3.5d). Other characteristic bands at 1637, 1156, 1079, 1024 and 931 cm\textsuperscript{-1} indicated that no degradation of the other components took place during desulphation.

Absence of the band at 897 cm\textsuperscript{-1} confirmed that there was no pyruvate acetal substitution on the galactose residues among the galactans of \textit{Sarconema scinaioides} (Chiovitti \textit{et al}.1997). The presence of sulphate group at O-6 of 4-linked galactose units of \(\nu\)-carrageenan was observed at 869 cm\textsuperscript{-1} (Tuvikene \textit{et al}. 2010). Therefore, the presence of IR bands at 931, 853 and 806 cm\textsuperscript{-1} confirmed that galactan obtained

<table>
<thead>
<tr>
<th>Extract</th>
<th>Molecular weights (KDa)</th>
<th>Polydispersity</th>
</tr>
</thead>
<tbody>
<tr>
<td>SF\textsubscript{Crude}</td>
<td>207.81</td>
<td>948.62</td>
</tr>
<tr>
<td>SF\textsubscript{AM}</td>
<td>341.74</td>
<td>1020.94</td>
</tr>
<tr>
<td>SF\textsubscript{Des}</td>
<td>3.05</td>
<td>10.000</td>
</tr>
<tr>
<td>SS\textsubscript{Native}</td>
<td>193.43</td>
<td>953.10</td>
</tr>
<tr>
<td>SS\textsubscript{Crude}</td>
<td>423.30</td>
<td>1247.53</td>
</tr>
<tr>
<td>SS\textsubscript{AM}</td>
<td>83.867</td>
<td>505.38</td>
</tr>
<tr>
<td>SS\textsubscript{Des}</td>
<td>75.12</td>
<td>149.80</td>
</tr>
</tbody>
</table>
from *Sarconema scinaoides* was indeed β-carrageenan along with its precursor ν-carrageenan (Matsuhiro and Rivas 1993; Chiovitti *et al.* 1997; Pereira *et al.* 2003; Yermak *et al.* 2006).

**NMR analysis of sulphated galactan of *Sarconema filiforme***

The $^1$H NMR spectrum of SF$_{Crude}$ exhibited the anomeric proton signals (Figure I.3.6a) in the range of 4.49-4.59 ppm (assigned to H-1 of β-D-Gal residue of α, τ and pyruvated α carrageenans respectively), at 5.39 ppm (H-1 of α-D-AnGal residue of τ carrageenan) and at 5.15 ppm (H-1 of α-D-AnGal residue of α carrageenan) (Pereira *et al.* 2003; Yermak *et al.* 2006; Kacuráková *et al.* 2000). The resonance at 3.31 ppm was assigned to the methyl protons of 6-O-methyl Gal (Falshaw *et al.* 2003), and the one at 1.33 ppm to methyl hydrogens of pyruvated α carrageenan (Pereira *et al.* 2003). The proton resonances in the range of 3.32-4.81 ppm were attributed to the remaining methylene and methine hydrogens of the carrageenan units.

The $^{13}$C NMR chemical shifts of SF$_{Crude}$ (Figure I.3.6b) were comparable to those of carrageenans reported earlier (Gorin and Mazurek 1975; Chiovitti *et al.* 1997 and 2004; Kacuráková *et al.* 2000; Van de Velde *et al.* 2002 and 2004; Yermak *et al.* 2006; Andriamanantoanina *et al.* 2007). Anomeric carbon resonance pairs at 102.3 & 92.1, 103.1 & 92.8 and 105.8 & 95.3 ppm were assigned to the diads belonging to pyruvated α, α and τ carrageenans respectively (Figure I.3.6b). The carbon resonance at 59.7 ppm was assigned to the methylated C-6 of the 3-linked galactose (Van de Velde *et al.* 2002 and 2004; Chiovitti *et al.* 2004; Andriamanantoanina *et al.* 2007). Additional $^{13}$C NMR resonances at 176.6, 101.0 & 26.3 ppm indicated the presence of carboxyl, acetal and methyl carbons of the pyruvate unit. Three weak signals at 67.7, 67.4 and 66.2 ppm were assigned to C-4, C-5 and C-6 of the 3-linked pyruvated galactose unit (Chiovitti *et al.* 2004). The presences of pyruvate groups in many red seaweeds polysaccharides such as in agar and carrageenan type polysaccharides have been reported in the literature (Usov *et al.* 1980; Guibet *et al.* 2008). The correlated C/H signals (59.7/3.31 ppm) of methyl carbon of 6-O-methyl galactose and CH$_3$ group (at 26.3/1.33 ppm) of pyruvated α-carrageenan were confirmed through their cross peak identification using HSQC spectra (Figure I.3.7b; Martone *et al.* 2010). The $^1$H-$^1$H COSY spectrum was also recorded which showed 15 H/H cross peaks. The latter could not be assigned because of the complex nature of the proton NMR spectrum (Figure I.3.7a).
NMR analysis of sulphated galactan of *Sarconema scinaioides*

The $^1$H NMR spectrum of SS$_{AM}$ exhibited the anomeric proton signals at 4.85, 5.33 and 5.50 ppm of G4S, DA2S (of iota carrageenan) and D2S6S (of $\nu$ carrageenan) respectively (Figure I.3.9a; Villanueva *et al.* 2009, Pereira and Van de Velde 2011). The signal at 3.43 ppm was assigned to the methyl proton of 6-O-methyl Gal. The proton resonances in the range of 3.63-4.91 ppm were attributed to the remaining methylene and methine hydrogens of the carrageenan units. The $^{13}$C NMR resonances of SS$_{Native}$ and SS$_{Crude}$ were assigned to G4S and DA2S units of $\iota$-carrageenan respectively along with the presence of 6-O-Methyl residue attached to 3-linked galactose (Van de Velde *et al.* 2002; Figure I.3.8). In addition presence of floridean starch, a storage polymer of red seaweeds species, was also detected in the SS$_{Native}$ and assigned by $^{13}$C NMR (Figure I.3.8a). The $^{13}$C NMR resonances of SS$_{AM}$ were assigned and depicted in Figure I.3.9b. The chemical shifts were identical to those of $\iota$-carrageenan are in good agreement with the values reported earlier (Chiovitti *et al.* 1998, Usov 1998; Van de Velde *et al.* 2002 and 2004). Absence of $^{13}$C resonances at 176.49, 102.25 & 26.26 ppm indicated that there were no carboxyl, acetal and methyl carbons of the pyruvate unit (Chiovitti *et al.* 1998), which were present in the galactan of the same seaweed species reported earlier by Van de Velde *et al.* (2005).

The anomic and as well other proton signals of SS$_{AM}$ were assigned unambiguously from the $^1$H COSY NMR experiment. The correlations of protons of G4S-2,3; G4S-3,4; G4S-6,6'; DA2S-2,3 and DA2S-4,5 were deduced from the COSY spectrum (Figure I.3.10a). The $^{13}$C resonances of 6-O-Me residue of 3-linked galactose 4-sulphate was observed at 59.7 ppm. The correlation of C/H of 6-O-Me (59.7/3.23 ppm), G4S (C$_{I}$/H$_{I}$- 103/4.85 ppm) and DA2S (C$_{I}$/H$_{I}$- 92.8/5.33 ppm) as well as other C/H correlations were assigned unambiguously by HSQC analysis (Figure I.3.10b).

### I.3.3.3 Monosaccharide composition analyses

The retention times of alditol acetates of standard sugars and 3,6-AnGal, 6-O-Me Gal are already discussed in the section I.2.3.4. The GC-MS profile of alditol acetates revealed that SF$_{Crude}$, SF$_{AM}$ and SF$_{Des}$ were composed of Gal, 3,6-AnGal, 6-O-Me Gal and glucose in a mol % of 64.5:23.0:9.8:2.6 (SF$_{Crude}$), 59.6:29.8:7.6:1.6 (SF$_{AM}$) and 68.4:19.3:10.2:3.1 (SF$_{Des}$) respectively (Table I.3.1). Alkaline treatment enhanced the 3,6-AnGal content by 7 mol % while it lowered the sulphate content by ~2% in SF$_{AM}$. Higher mol % of 6-O-Me Gal was observed in Indian sample compared
with the Australian one, indicating compositional variation in above polysaccharides of the same seaweed species collected from different geographical locations.

The GC-MS profile of alditol acetates of $SS_{\text{Crude}}$, $SS_{\text{AM}}$ and $SS_{\text{Des}}$ revealed that sulphated polysaccharides were mainly composed of Gal, 3,6-AnGal, 6-O-Me Gal along with trace amount of xylose in various molar proportions (Table I.3.1). The native carrageenan ($SS_{\text{Native}}$) contained Gal, 3,6-AnGal, 6-O-Me Gal along with glucose (22.5 mole %), the latter being solely due to floridean starch (Table I.3.1, Falshaw and Furneaux 1998; Freile-Pelegrín et al. 2011). Alkali modification of $SS_{\text{Crude}}$ resulted in an increase of 3,6-AnGal content by 4.0 mole %, by cyclization of D2S,6S ($\nu$-carrageenan unit) in to DA2S units, associated with the increase (ca. 2.1%) in sulphate content as well as with a decrease in 6-O-methyl galactose content, presumably because of leach out of polysaccharides during alkaline modification. The ratios of AnGal: Gal, which is assumed to be 1:1 for ideal carrageenan, for $SS_{\text{Crude}}$ and $SS_{\text{AM}}$ were considerably less, which may be due to the incomplete recovery of AnGal by reductive hydrolysis of $\iota$-carrageenan having sulphate ester substitution at O-2 of the AnGal residues, resulting in higher amount of galactose residues (cf. Stevenson and Furneaux 1991; Falshaw et al. 1996). There was significant amount of 6-O-Me Gal units in all the samples (Table I.3.1).

### I.3.3.4 Linkage analysis of $SF_{\text{Crude}}$, $SF_{\text{AM}}$ and $SF_{\text{Des}}$

Linkage analysis of $SF_{\text{Des}}$ showed the presence of 4-linked 3,6-AnGal, 3,4,6-linked Gal, 4-linked Gal, 3-linked Gal and 4,6-linked Gal in a mol % of 36.9: 26.1: 1.8: 27.0: 8.6 along with a trace amount of terminal galactose (Table I.3.4). The presence of 3,4,6-linked Gal was due to 3-linked Gal having pyruvate acetal linked between C-4 and C-6 of $\alpha$-carrageenan (Chiovitti et al. 1998). Higher mol % of 3,4,6-linked Gal indicated that the polysaccharide was highly pyruvated (Table I.3.4).

To determine $\alpha$, $\iota$ and pyruvated carrageenan contents and the position of sulphate ester, linkage analysis of $SF_{\text{Crude}}$ and $SF_{\text{AM}}$ were done. In linkage analysis of $SF_{\text{Crude}}/SF_{\text{AM}}$, 3-, 3,4- and 3,4,6- linked Gal were obtained in a mol % of 17.6: 18.8: 25.3 for $SF_{\text{Crude}}$ and 15.5: 14.6: 22.8 mol% for $SF_{\text{AM}}$ along with a common 2,4-linked 3,6 AnGal (27.1 for $SF_{\text{Crude}}$ and 32.1 mol% for $SF_{\text{AM}}$) residues, which were attributed to $\alpha$, $\iota$ and pyruvated $\alpha$ carrageenans respectively. The 4,6-linked Gal residue in $SF_{\text{Des}}$ and $SF_{\text{Crude}}/SF_{\text{AM}}$ were obtained in comparable mole % values indicating thereby that these did not bear sulphate ester at C-6 (Table I.3.4). Since only 2,4-linked 3,6-AnGal was present in $SF_{\text{Crude}}/SF_{\text{AM}}$, it is obvious that all the 3,6-AnGal were sulphated at C-2 (Table I.3.4). The proportions of $\alpha$- and $\iota$-carrageenan were almost similar while that
of pyruvated α carrageenan was higher (cf. Table I.3.4). It may be noted that significant amount of 4,6-linked Gal was present in linkage analysis of SF<sub>Des</sub>, SF<sub>Crude</sub> and SF<sub>AM</sub> in mol% of 8.6, 9.1 and 8.2 respectively (cf. Table I.3.4). This may be ascribed either to (i) the terminal galactose units bearing pyruvated acetal group involving C-4 and C-6 or to (ii) 4- and 6-linked galactose units not having pyruvated acetal moiety. Besides this, other variously linked minor (<2 mole %) Gal units emerged from the linkage analysis of SF<sub>Crude</sub>/SF<sub>AM</sub> which were 2,3,6-, 2,3,4-, and 2,3-linked Gal residues.

I.3.3.5 Linkage analysis of SS<sub>Native</sub>, SS<sub>AM</sub> and SS<sub>Des</sub>

Linkage analysis of SS<sub>Native</sub> and SS<sub>AM</sub> showed the presence of 2,4-linked 3,6-AnGal; 2,4,6-linked Gal; 3,4-linked Gal; 4-linked Gal; 3-linked Gal units in various molar proportions (Table I.3.4). The main linked units were 2,4-linked 3,6-AnGal and 3,4-linked-Gal accounted for DA2S and G4S of ι-carrageenan units respectively. The presence of 2,4,6-linked Gal were accounted for D2S,6S units of ν-carrageenan. The presence of significant amount of 4-linked glucose (24.2 mole %) in SS<sub>Native</sub> was due to floridean starch, which was removed by α-amylase treatment. The other linked galactose units were 4-linked Gal and 3-linked Gal (< 5 moles %, Table I.3.4).

Table I.3.4 Linkages analysis of galactans of Sarconema filiforme and Sarconema scinaioides

<table>
<thead>
<tr>
<th>Deduced linkage</th>
<th>Sarconema filiforme</th>
<th>Sarconema scinaioides</th>
</tr>
</thead>
<tbody>
<tr>
<td>→2,4)- 3,6 AnGalp (1→</td>
<td>27.1</td>
<td>32.1</td>
</tr>
<tr>
<td>→4)- 3,6 AnGalp (1→</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>→2,4,6)-Galp (1→</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td>→2,3,4)-Galp (1→</td>
<td>1.4</td>
<td>1.9</td>
</tr>
<tr>
<td>→2,3,6)-Galp (1→</td>
<td>1.8</td>
<td>1.5</td>
</tr>
<tr>
<td>→3,4,6)-Galp (1→</td>
<td>25.3</td>
<td>22.8</td>
</tr>
<tr>
<td>→2,3)-Galp (1→</td>
<td>0.9</td>
<td>1.1</td>
</tr>
<tr>
<td>→3,4)-Galp (1→</td>
<td>18.8</td>
<td>14.6</td>
</tr>
<tr>
<td>→4,6)-Galp (1→</td>
<td>9.1</td>
<td>8.2</td>
</tr>
<tr>
<td>→3)-Galp (1→</td>
<td>17.6</td>
<td>15.5</td>
</tr>
<tr>
<td>→4)-Galp (1→</td>
<td>1.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Terminal Galp (1→</td>
<td>tr</td>
<td>tr</td>
</tr>
<tr>
<td>→4)-Glp (1→</td>
<td>---</td>
<td>---</td>
</tr>
</tbody>
</table>

---: Absent

The positions of sulphate ester group were determined by the linkages analysis of desulphated derivative SS<sub>Des</sub>. Linkage analysis of SS<sub>Des</sub> showed the presence of 4-linked 3,6-AnGal; 2,4-linked 3,6-AnGal; 2,4,6-linked Gal; 3,4-linked Gal; 4-linked Gal and 3-linked Gal units (Table I.3.4). The presence of higher amount of 4-linked...
3,6-AnGal and 3-linked Gal contents in SS$_D$ suggested that all the 4-linked 3,6-AnGal units were sulphated at O-2 and 3-linked Gal were sulphated at O-4, as found in DA2S and G4S units of the t-carrageenan. The presence of 4-linked Gal units was attributed to the desulphated derivative of D2S,6S units of ν-carrageenan. Other linked minor sugar units were 2,4-linked 3,6-AnGal and 3,4-linked Gal (< 4 mole %; Table I.3.4).

I.3.4 SUMMARY

On the basis of $^1$H, $^{13}$C-NMR spectra and linkage analysis, it is proposed that the sulphated galactan of Sarconema filiforme of Indian waters is a hybrid/combination polysaccharide composed of α, pyruvated α and methylated α and i carrageenans. The carrageenan contents and the relative proportions of their constituent monosaccharides e.g. α, i, pyruvated α, 6-O-Me Gal and galactose were different from those of Sarconema filiforme of Australian waters (Chiovitti et al. 1998). This work updates the earlier report of carrageenan of this seaweed species of Indian waters as i carrageenan only on the basis of IR studies (Parekh et al. 1988; Rajasulochana and Gunasekaran 2009).

The carrageenan of Sarconema scinaioides of Indian waters consisted predominantly of iota-carrageenan along with a small amount of its precursor ν-carrageenan. No κ-carrageenan or pyruvated units were detected in this Indian seaweeds species unlike the carrageenan of Sarconema scinaioides reported by Van de Velde et al. (2005). To our knowledge this is the first report of the sole occurrence of iota-carrageenan along with its precursor (ν) in Sarconema scinaioides. The results of this study would be useful in bioprospecting of carrageenophytes. These results have been published (Kumar et al. 2011, 2012).

I.3.5 REFERENCES


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Oza, M.D., Mehta, G.K., Kumar, S., Meena, R., Siddhanta, A.K. 2011. *Phycological Research* 59, 244-249.


www.algaebase.org

http://www.ccrc.uga.edu/specdb/ms/pmaa/pframe.html
Figure I.3.1: Flow behavior of SF_{Crude} (2.5 % w/v) in water and salt solutions (1% w/v)

Figure I.3.2: Flow behavior of SS_{Crude} and iota carrageenan (1 % w/v) in salt solutions (1% w/v)
Figure 1.3.3: Gel permeation chromatograms of (a) SF$_{\text{Crude}}$, (b) SF$_{\text{AM}}$, (c) SF$_{\text{Des}}$ (d) SS$_{\text{Native}}$ (e) SS$_{\text{Crude}}$ (f) SS$_{\text{AM}}$ and (g) SS$_{\text{Des}}$
Figure I.3.4: FTIR spectra (a) SF\textsubscript{Crude}, (b) SF\textsubscript{AM} and (c) SF\textsubscript{Des}

Figure I.3.5: FTIR spectra (a) SS\textsubscript{Native}, (b) SS\textsubscript{Crude}, (c) SS\textsubscript{AM} and (d) SS\textsubscript{Des}
Figure I.3.6: (a) $^1$H and (b) $^{13}$C NMR Spectra of SFCrude
Figure I.3.7: (a) 2D $^1$H-$^1$H COSY and (b) $^1$H-$^{13}$C HSQC NMR of SF$_{\text{Crude}}$. 
Figure I.3.8: $^{13}$C NMR spectrum of (a) SS$_{\text{Native}}$ and (b) SS$_{\text{Crude}}$
Figure I.3.9: (a) $^1$H and (b) $^{13}$C NMR spectrum of SS$_{AM}$
Figure I.3.10: (a) 2D $^1$H-$^1$H COSY and (b) $^1$H-$^{13}$C HSQC NMR of SS$_{AM}$
Figure I.3.11: GC-MS analysis of galactan of *Sarconema filiforme* (a) SF<sub>Crude</sub>, (b) SF<sub>AM</sub> and (c) SF<sub>Des</sub>
<table>
<thead>
<tr>
<th>Peak#</th>
<th>R. Time</th>
<th>Area %</th>
<th>Name</th>
<th>Base m/z</th>
<th>Base Int.</th>
<th>Mole %</th>
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Figure I.3.11: Contd.
**Figure I.3.11:** GC-MS analysis of galactan of *Sarconema filiforme* (a) SF<sub>Crude</sub>, (b) SF<sub>AM</sub> and (c) SF<sub>Des</sub>

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**Figure I.3.12:** GC-MS analysis of galactan of *Sarconema scinaoides* (a) SS<sub>Native</sub>, (b) SS<sub>Crude</sub>, (c) SS<sub>AM</sub> and (d) SS<sub>Des</sub>
Table 1.4.1.2: Peak Report TIC

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Figure I.3.12: Contd.
Figure I.3.12: Contd.
Peak Report TIC

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Figure I.3.12: GC-MS analysis of galactan of *Sarconema scinaoides* (a) SS_{Native}, (b) SS_{Crude}, (c) SS_{AM} and (d) SS_{Des}
Figure I.3.13: GCMS of PMAA of *Sarconema filiforme* (a) SF\textsubscript{Crude}, (b) SF\textsubscript{AM} and (c) SF\textsubscript{Des}
### Table 1

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<th>Linked Sugar</th>
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Figure I.3.13: GCMS of PMAA of *Sarconema filiforme* (a) SF\textsubscript{Crude}, (b) SF\textsubscript{AM} and (c) SF\textsubscript{Des}
### Figure I.3.14: GC-MS analysis of PMAA of *Sarconema scinaoides* (a) SS<sub>Native</sub>, (b) SS<sub>AM</sub> and (c) SS<sub>Des</sub>

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### Peak Report TIC

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### Figure I.3.14: Contd.
**Peak Report TIC**

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**Figure I.3.14:** GC-MS analysis of PMAA of *Sarconema scinioides* (a) SS<sub>Native</sub>, (b) SS<sub>AM</sub> and (c) SS<sub>Des</sub>