In the Trás-os-Montes region, the use of infusions of *Fraxinus angustifolia* dried leaves as a protection against high levels of cholesterol, blood pressure and uric acid is widespread. Pectic polysaccharides isolated from the infusions of some medicinal plants have been reported as biologically active [1,2]. Pectic polysaccharides have been described as structurally complex polymers, exhibiting different polymeric building blocks: homogalacturonans (HG), rhamnogalacturonans-I (RG-I), rhamnogalacturonans-II (RG-II) and xylogalacturonans (XG) [3]. The backbone of RG-I can be partly substituted with various side chains, such as arabinans, type-I and type-II arabinogalactans (AG-I and AG-II). The aim of this work is to provide a first insight of the nature of the pectic polysaccharides present in the infusions of *F. angustifolia* dried leaves.

### Methodologies

I.- Preparation of the High Molecular Weight Material (HMWM) and Ethanol Precipitation

*Fraxinus angustifolia* dried leaves in boiling water (0.05-0.07 mg/mL) during a total of 4h (2 h + 2 h)

**Scheme I** - Preparation of the HMWM.

- 75% Ethanol Solutions Insoluble HMWM
- 50% Ethanol Solutions Insoluble HMWM
- 75% Ethanol Solutions Soluble HMWM

**Ethanol Precipitation of the HMWM**

- 75% Ethanol Solutions Soluble HMWM
- 75% Ethanol Solutions Insoluble HMWM
- 50% Ethanol Solutions Insoluble HMWM
- 75% Ethanol Solutions Insoluble HMWM

**II.- Anion Exchange Chromatography (DEAE-Sepharose FF AEC)**

*Phosphate buffer (pH 6.0)*

**Scheme III** - Anion Exchange Chromatography Procedure.

- Elution profile of the fraction $E_{B1,1}$
- Elution profile of the fraction $E_{B1,2}$
- Elution profile of the fraction $E_{B1,3}$
- Elution profile of the fraction $E_{B1,4}$
- Elution profile of the fraction $E_{B1,5}$

### Results

#### Table I- Material yield, total sugars and monomeric composition of HMWM.1 and HMWM.2.

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield (%)</th>
<th>Total Sugars (%)</th>
<th>Monomeric Composition, (molar %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HMWM.1</td>
<td>90.0</td>
<td>12.0</td>
<td>Rha, Ara, Xyl, Man, Gal, Glc, UA</td>
</tr>
<tr>
<td>HMWM.2</td>
<td>90.5</td>
<td>8.0</td>
<td>Rha, Ara, Xyl, Man, Gal, Glc, UA</td>
</tr>
</tbody>
</table>

**Pectic polysaccharides**

**Table II- Material yield, total sugars and monomeric composition of the various fractions obtained from ethanol precipitation of HMWM.1 and HMWM.2.**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield (%)</th>
<th>Total Sugars (%)</th>
<th>Monomeric Composition, (molar %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{B1,1}$</td>
<td>49.0</td>
<td>61.0</td>
<td>Rha, Ara, Xyl, Man, Gal, Glc, UA</td>
</tr>
<tr>
<td>$E_{B1,2}$</td>
<td>16.9</td>
<td>55.8</td>
<td>Rha, Ara, Xyl, Man, Gal, Glc, UA</td>
</tr>
<tr>
<td>$E_{B1,3}$</td>
<td>24.1</td>
<td>84.7</td>
<td>Rha, Ara, Xyl, Man, Gal, Glc, UA</td>
</tr>
<tr>
<td>$E_{B1,4}$</td>
<td>34.5</td>
<td>33.0</td>
<td>Rha, Ara, Xyl, Man, Gal, Glc, UA</td>
</tr>
<tr>
<td>$E_{B1,5}$</td>
<td>26.3</td>
<td>38.8</td>
<td>Rha, Ara, Xyl, Man, Gal, Glc, UA</td>
</tr>
</tbody>
</table>

**Pectic polysaccharides “enriched” in HG regions**

**Pectic polysaccharides “enriched” in RG-I regions**

**Is the AG-II region biologically active, as its structure is similar to that reported by Sakurai et al. in 1998?**

**Table III- Material yield, total sugars and monomeric composition of the major fractions obtained from DEAE-Sepharose FF AEC (fractions obtained from HMWM 3 showed similar results).**

<table>
<thead>
<tr>
<th>Fraction</th>
<th>Yield (%)</th>
<th>Total Sugars (%)</th>
<th>Monomeric Composition, (molar %)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_{B1,1}$</td>
<td>45.0</td>
<td>76.7</td>
<td>Rha, Ara, Xyl, Man, Gal, Glc, UA</td>
</tr>
<tr>
<td>$E_{B1,1}$</td>
<td>45.6</td>
<td>76.7</td>
<td>Rha, Ara, Xyl, Man, Gal, Glc, UA</td>
</tr>
<tr>
<td>$E_{B1,2}$</td>
<td>64.9</td>
<td>44.1</td>
<td>Rha, Ara, Xyl, Man, Gal, Glc, UA</td>
</tr>
</tbody>
</table>

**Pectic polysaccharides “enriched” in DG domains**

**Pectic polysaccharides “enriched” in RG domains**

More than 60% of the recovered HMWM was collected in one or two fractions, suggesting some structural homogeneity among the pectic polysaccharides with the same ethanol solubility characteristics present in the *Fraxinus angustifolia* dried leaves infusions.

- Although additional studies need to be performed in order to fully elucidate the detailed structure of the pectic polysaccharides present and to assess their biological activity, linkage analysis suggested the presence of AG-II, which exhibited structures similar to some previously reported as biologically active.

**References**


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