

# Monomeric Composition and Linkage Analysis of the Polysaccharides Present in the Infusions of *Fraxinus angustifolia* Leaves

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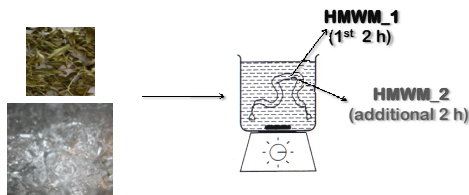
Introduction

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.

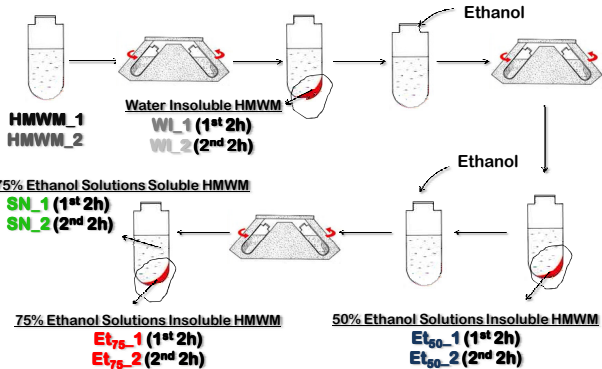
## Methodologies

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



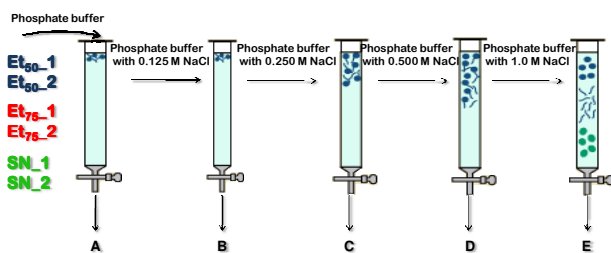
*Fraxinus angustifolia* dried leaves in boiling water (0.05-0.07 mg/mL) during a total of 4h (2 h + 2 h)

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

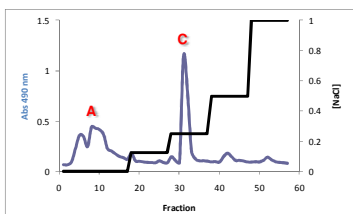


#### Scheme II- Ethanol Precipitation of the HMWM's.

### II- Anion Exchange Chromatography (DEAE-Sephacrose FF AEC)



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



Elution profile of the fraction **Et<sub>75-1</sub>**.

## Results

**Table I-** Material yield, total sugars and monomeric composition of HMWM<sub>1</sub> and HMWM<sub>2</sub>.

Fraction	Yield <sup>*</sup> (%)	Total Sugars (%)	Monomeric Composition, (molar %)						
			Rha	Ara	Xyl	Man	Gal	Glc	UA
HMWM <sub>1</sub>	2.2	57.0	4.2	7.6	1.6	2.7	5.3	11.2	67.3
HMWM <sub>2</sub>	1.6	76.1	2.5	11.6	1.8	1.5	5.5	5.3	71.7

\* expressed relatively to the quantity of vegetable material used in the preparation of the infusion

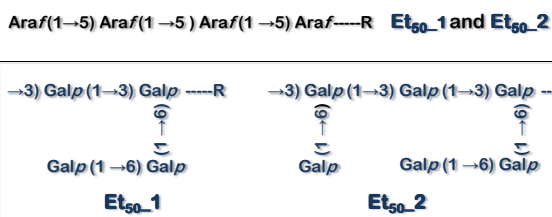
The additional extraction of 2 h allowed to extract more **73%** of HMWM

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

Fraction	Yield <sup>*</sup> (%)	Total Sugars (%)	Monomeric Composition, (molar %)						
			Rha	Ara	Xyl	Man	Gal	Glc	UA
Et <sub>50-1</sub>	48.6	81.0	2.1	3.5	1.6	0.5	3.2	3.0	86.3
Et <sub>50-2</sub>	49.6	90.1	1.6	4.3	1.5	0.2	2.9	1.1	88.5
Et <sub>75-1</sub>	16.9	55.8	3.4	11.2	2.6	2.6	12.8	9.8	57.8
Et <sub>75-2</sub>	24.1	84.7	2.5	10.6	2.6	1.1	9.5	4.1	69.8
SN <sub>1</sub>	34.5	33.0	8.4	17.0	1.2	11.0	5.3	33.9	23.3
SN <sub>2</sub>	26.3	38.8	5.3	35.7	1.2	8.0	4.5	22.3	23.1

\* expressed relatively to the quantity of HMWM submitted to ethanol precipitation

#### Possible arabinan and galactan structures present in the pectic polysaccharides "enriched" in HG regions



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

**AG-II** region reported as biologically active (Sakurai *et al.*, 1998)

**Table II-** Material yield, total sugars and monomeric composition of the major fractions obtained from DEAE-Sephacrose FF AEC (fractions obtained from HMWM<sub>2</sub> showed similar results).

Fraction	Yield <sup>*</sup> (%)	Total Sugars (%)	Monomeric Composition, (molar %)						
			Rha	Ara	Xyl	Man	Gal	Glc	UA
Et <sub>50-1C</sub>	88.0	86.0	1.6	3.8	1.5	0.0	3.4	0.9	88.9
Et <sub>75-1A</sub>	45.0	76.7	0.7	8.0	3.1	4.2	13.6	6.8	63.6
Et <sub>75-1C</sub>	45.6	70.3	3.9	14.3	1.2	0.5	13.7	3.2	63.2
SN <sub>1A</sub>	64.9	44.1	2.0	25.5	1.6	18.4	7.2	26.2	19.1

\* expressed relatively to the recovered HMWM

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.

Conclusions

- Infusions from *Fraxinus angustifolia* contained 3.8% of high molecular weight material comprising a mixture of polysaccharides, including pectic polysaccharides.
- Ethanol precipitation allowed to fractionated pectic polysaccharides according to uronic acid content, possibly related to the proportion of HG and RG-I regions present in their composition.

- 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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