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Structural features and macrophage immunostimulatory activity of the polysaccharides present from *Fraxinus angustifolia* and *Mentha suaveolens* hot water extracts

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Introduction

The dried leaves of *Fraxinus angustifolia* and the dried shoots of *Mentha suaveolens* are used by the population of the Trás-os-Montes, which is a region located in the northeast of Portugal, to prepare hot water extracts due to their therapeutic properties, particularly against rheumatism, high blood pressure and high levels of uric acid [1]. Recently, the macrophage immunomodulation and therapeutic potential of pectic polysaccharides from diverse plant hot water extracts has been highlighted [2,3].

Objectives

This work aims to provide a structural characterization of the polysaccharides present in these hot water extracts and will also evaluate their possible macrophage immunostimulatory activity, which might contribute for the therapeutic properties reported for these hot water extracts.

Methods


100°C/(2h+2h)

F. angustifolia HWE — *M. suaveolens* HWE

Dialysis (MWCO 12-14 kDa)

High Molecular Weight Material (HMWM)

Ethanol precipitation

WI_{ppt} | Et_{50} | Et_{75} | SN

Anion exchange chromatography (AEC)

Buffer (pH 6.5) — A | 0.125 M NaCl — B | 0.250 M NaCl — C | 1.0 M NaCl — D

Fractionation scheme

Analytical techniques

- Sugar and linkage composition of the fractions was performed using alditol acetates GC-FID analysis and partially methylated alditol acetates GC-MS analysis, respectively;
- Immunostimulatory activity was evaluated through the measurement of the nitrite (NO) production by macrophages (RAW 264.7 macrophage cell line).

Results

1. Ethanol precipitation

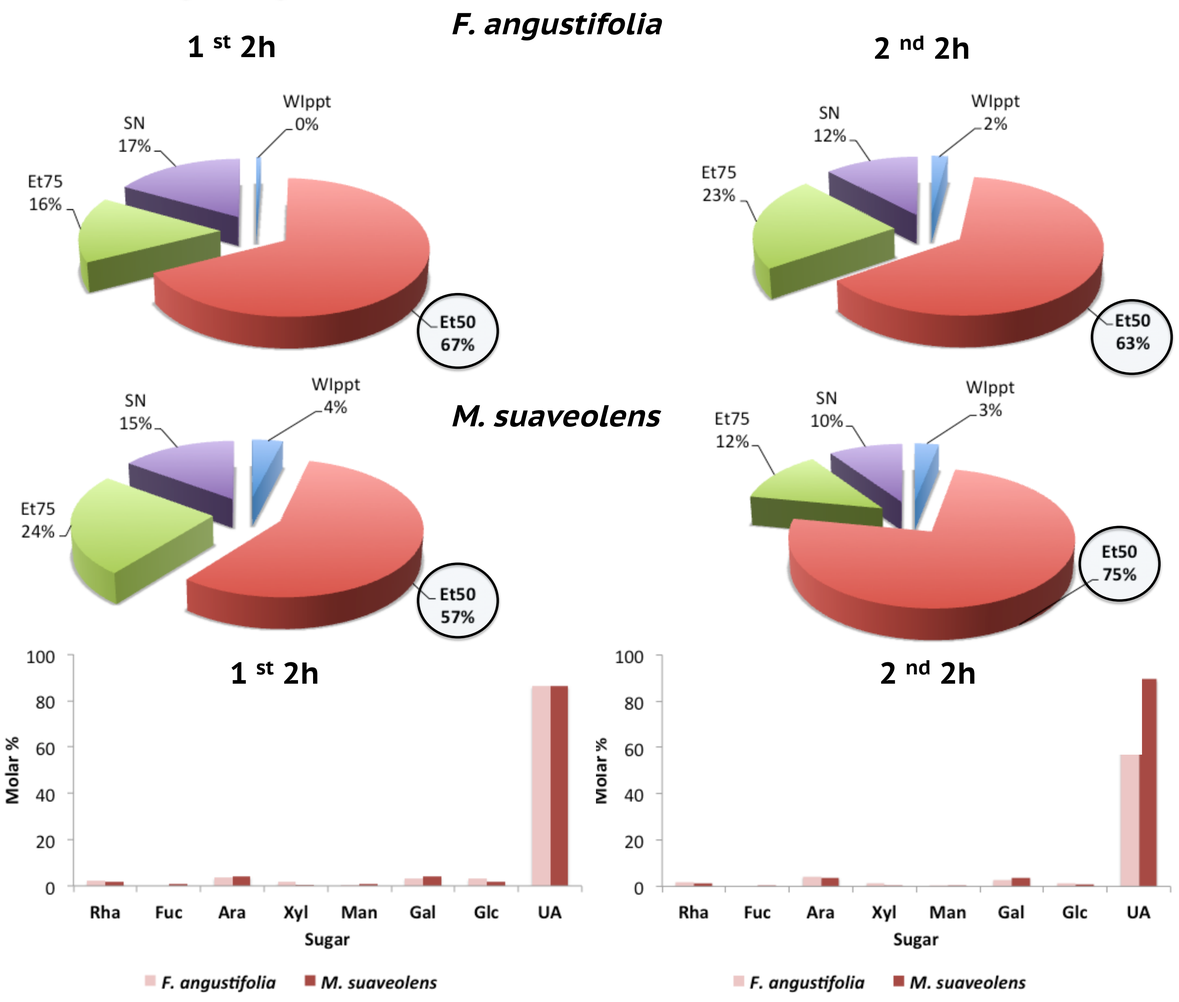
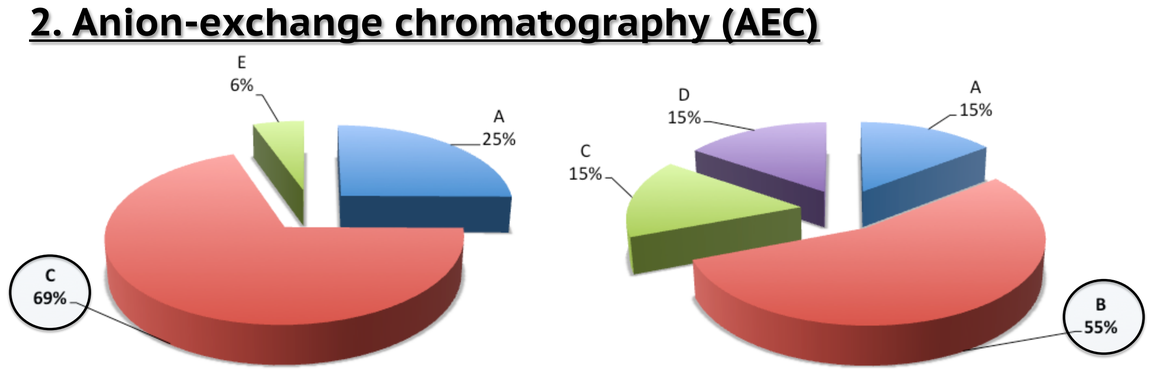


Figure 1-Polysaccharide content, expressed as mass percentage of the total polysaccharides recovered in the various fractions obtained through ethanol precipitation of the HMWM from the 1st and 2nd 2h, and sugar composition of the Et50 fractions (Rha-rhamnose; Fuc-fucose; Ara-arabinose; Xyl-xylose; Man-mannose; Gal-galactose; Glc-glucose; UA-uronic acid).

Results

2. Anion-exchange chromatography (AEC)



F. angustifolia (Et_{50} 1st 2h) — *M. suaveolens* (Et_{50} 1st 2h)

Figure 2- Polysaccharide content of the various fractions obtained through AEC of the Et_{50} fractions from the 1st 2h, expressed as mass percentage of the total polysaccharides recovered in the various fractions.

F. angustifolia

Linkage	Molar %	Linkage	Molar %
T-Rha*p*	2.3	T-Man*p*	0.0
2-Rha*p*	2.9	4-Man*p*	0.0
3-Rha*p*	1.3	2,4-Man*p*	0.0
2,4-Rha*p*	5.2	4,6-Man*p*	0.0
3,4-Rha*p*	2.2	**Total**	**0.0 (0.0)**
Total	**13.9[a] (13.2)[b]**		
		T-Gal*p*	8.3
T-Fuc*p*	2.7	2-Gal*p*	2.5
Total	**2.7 (0.7)**	3-Gal*p*	3.5
		4-Gal*p*	8.2
T-Ara*f*	5.3	6-Gal*p*	3.2
T-Ara*p*	1.4	3,6-Gal*p*	4.3
2-Ara*f*	0.7	**Total**	**30.0 (30.9)**
3-Ara*f*	2.1		
5-Ara*f*	20.6	T-Glc*p*	0.6
3,5-Ara*f*	5.2	3-Glc*p*	0.4
Total	**35.7 (37.5)**	4-Glc*p*	3.9
		2,4-Glc*p*	1.8
T-Xyl*p*	2.0	4,6-Glc*p*	1.3
2-Xyl*p*	0.3	**Total**	**8.0 (2.9)**
4-Xyl*p*	2.0		
2,3-Xyl*p*	1.2		
2,4-Xyl*p*	4.2		
Total	**9.7 (14.0)**		

M. suaveolens

Linkage	Molar %	Linkage	Molar %
T-Rha*p*	3.4	T-Man*p*	0.6
2-Rha*p*	2.2	4-Man*p*	0.0
3-Rha*p*	0.0	2,4-Man*p*	0.0
2,4-Rha*p*	2.9	4,6-Man*p*	0.0
3,4-Rha*p*	0.0	**Total**	**0.6 (1.7)**
Total	**8.5[a] (15.0)[b]**		
		T-Gal*p*	6.2
T-Fuc*p*	2.7	2-Gal*p*	0.0
Total	**2.7 (6.7)**	3-Gal*p*	3.4
		4-Gal*p*	5.6
T-Ara*f*	10.0	6-Gal*p*	8.6
T-Ara*p*	3.3	3,6-Gal*p*	14.3
2-Ara*f*	0.8	**Total**	**38.1 (30.0)**
3-Ara*f*	2.5		
5-Ara*f*	8.5	T-Glc*p*	1.8
3,5-Ara*f*	4.4	3-Glc*p*	1.2
Total	**29.5 (36.7)**	4-Glc*p*	2.3
		6-Glc*p*	0.9
		2,4-Glc*p*	2.6
T-Xyl*p*	2.6	4,6-Glc*p*	2.3
2-Xyl*p*	0.0	**Total**	**11.1 (8.3)**
4-Xyl*p*	6.0		
2,3-Xyl*p*	1.2		
2,4-Xyl*p*	0.9		
Total	**9.5 (3.3)**		

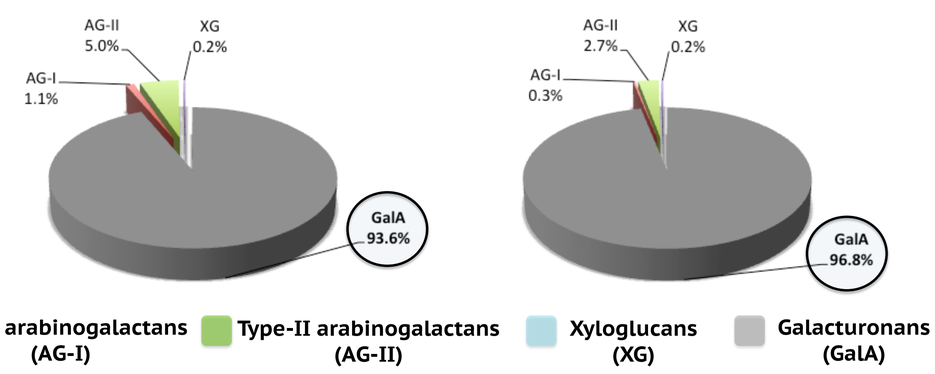


Figure 3- Deduced linkages and sugar composition, expressed as mass percentage, for the polysaccharides of Et_{50}C and Et_{50}B fractions from *F. angustifolia* and *M. suaveolens,* respectively ([a] linkage analysis; [b] sugar analysis).

- Most of the polysaccharides present in the HMWM precipitated in 50% aqueous ethanol solutions, and comprised very high proportions of UA residues, suggesting the presence of pectic polysaccharides;
- These polysaccharides were mostly recovered in the acidic fractions (Et50C, and Et50B, for F. angustifolia and M. suaveolens, respectively), and through methylation analysis it was also possible to detect minor proportions of neutral polysaccharides, particularly AG-II.

4. Macrophage immunostimulatory activity

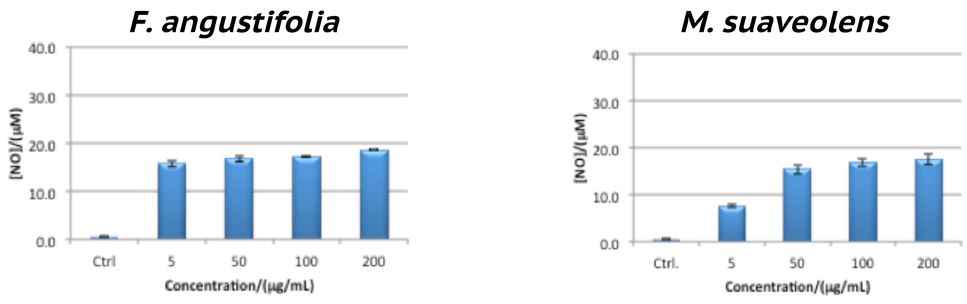


Figure 4- Macrophage immunostimulatory activity, expressed as NO production, of the Et50C and Et50B fractions from F. angustifolia and M. suaveolens, respectively.

- The macrophage NO production registered for all the assayed concentrations of both fractions evidence the immunostimulatory activity of the Et50C and Et50B fractions from F. angustifolia and M. suaveolens.

Conclusions

- The HWE from *F. angustifolia* dried leaves, and *M. suaveolens* dried shoots contain a mixture of polysaccharides, which comprised very high proportions of pectic polysaccharides together with minor proportions of AG-II, and that exhibited macrophage immunostimulatory activity.

References

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