

Structural characterization and macrophage immunostimulatory activity of polysaccharides present in the hot water extracts from *P. tridentatum* dried inflorescences

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Introduction

The hot water extracts (HWE) from *Pterospatum tridentatum* dried inflorescences are used in the northeastern portuguese region of Trás-os-Montes due to its therapeutic properties, namely against rheumatism, bladder, liver and kidney disorders [1]. The therapeutic properties attributed to hot water extracts from plants are often related with polysaccharide macrophage immunostimulatory activity [2].

Objectives

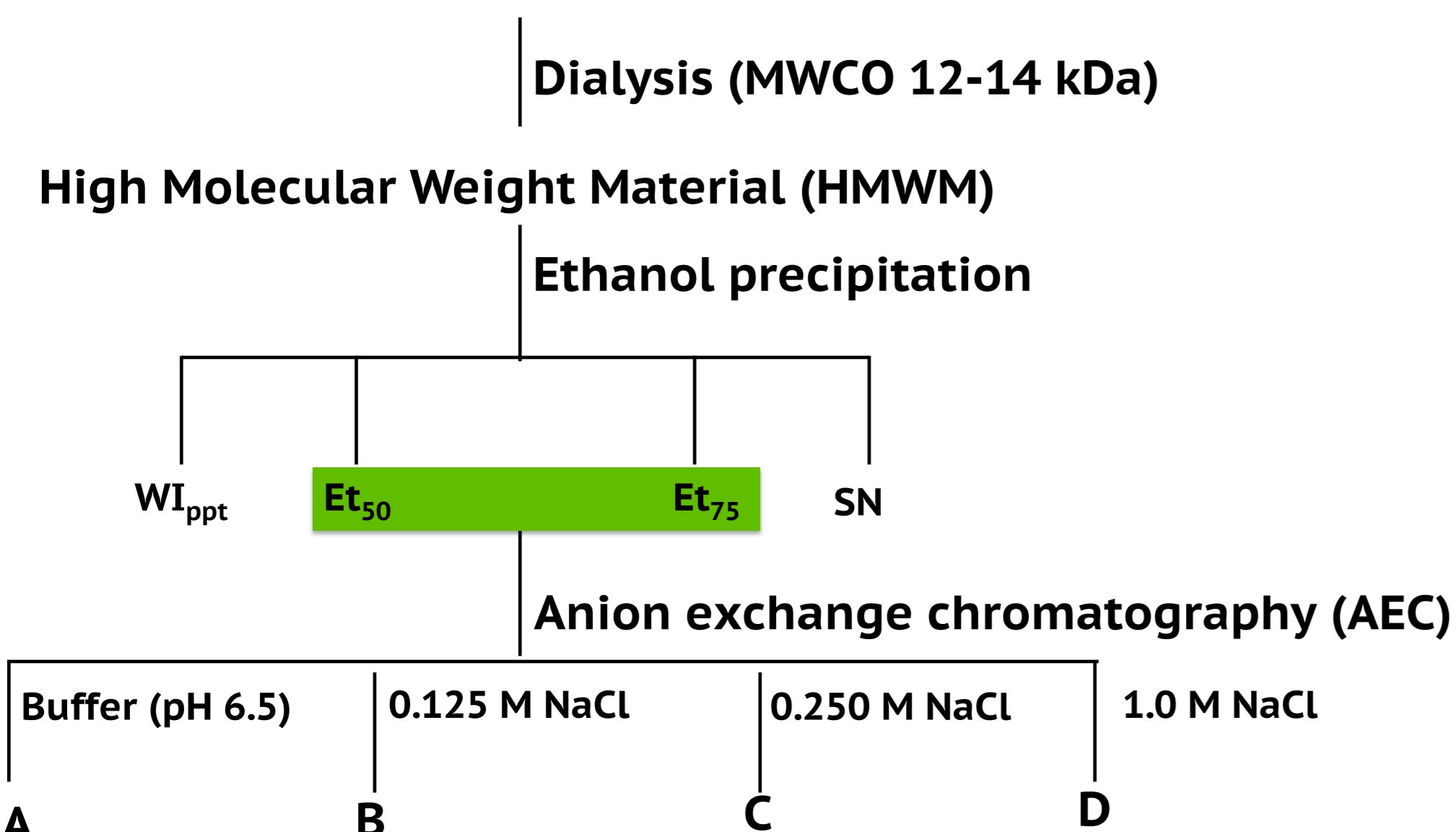
This work intends to identify the structural features of polysaccharides from *Pterospatum tridentatum* hot water extracts and to evaluate their putative macrophage immunostimulatory activity, which may contribute to their therapeutic properties.

Methods



P. tridentatum HWE (100°C/2h)

Fractionation scheme



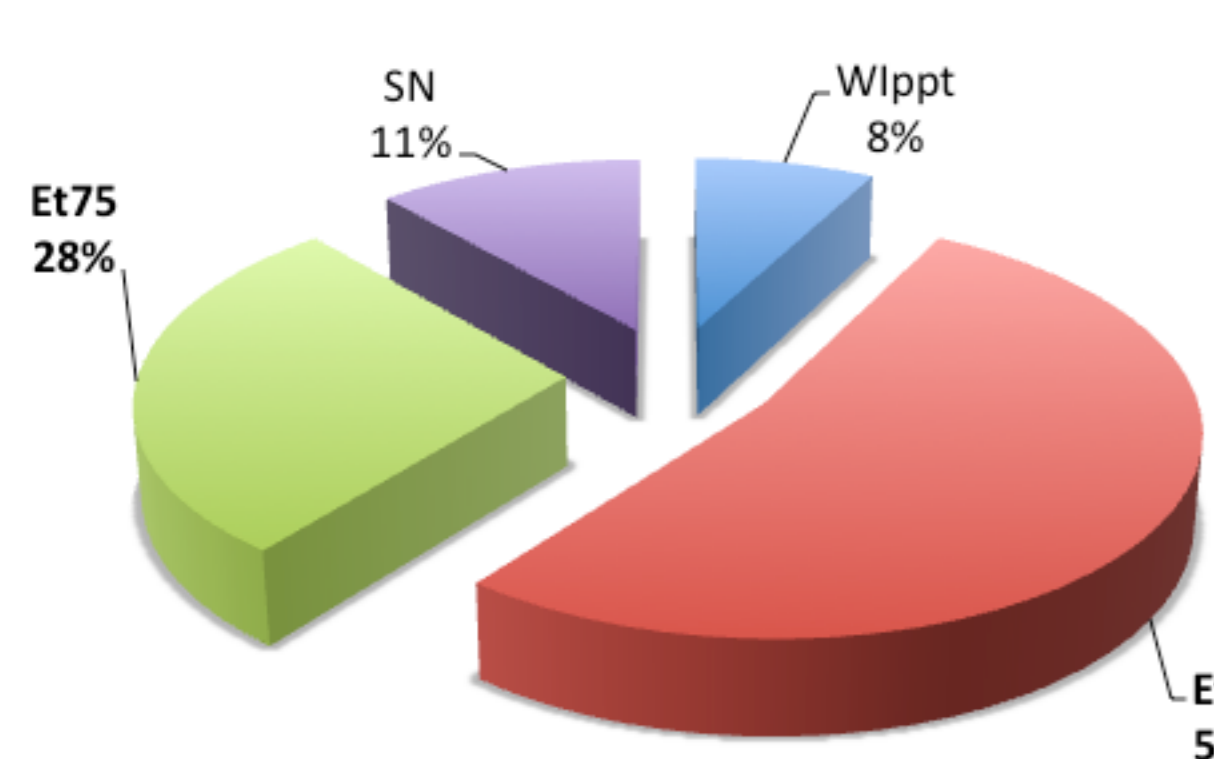
Analytical techniques

- Sugar and linkage composition of the fractions was performed using alditol acetates GC-FID analysis and partially methylated alditol acetates GC-MS analysis, respectively;
- The electrospray ionization mass spectrometry (ESI-MS) assays were performed in positive ion mode, after *endo*-mannanase treatment and size exclusion chromatography (SEC);
- Immunostimulatory activity was evaluated through the measurement of the nitrite (NO) production by macrophages (RAW 264.7 macrophage cell line).

Results

1. Ethanol precipitation

Polysaccharide Content



Sugar Composition

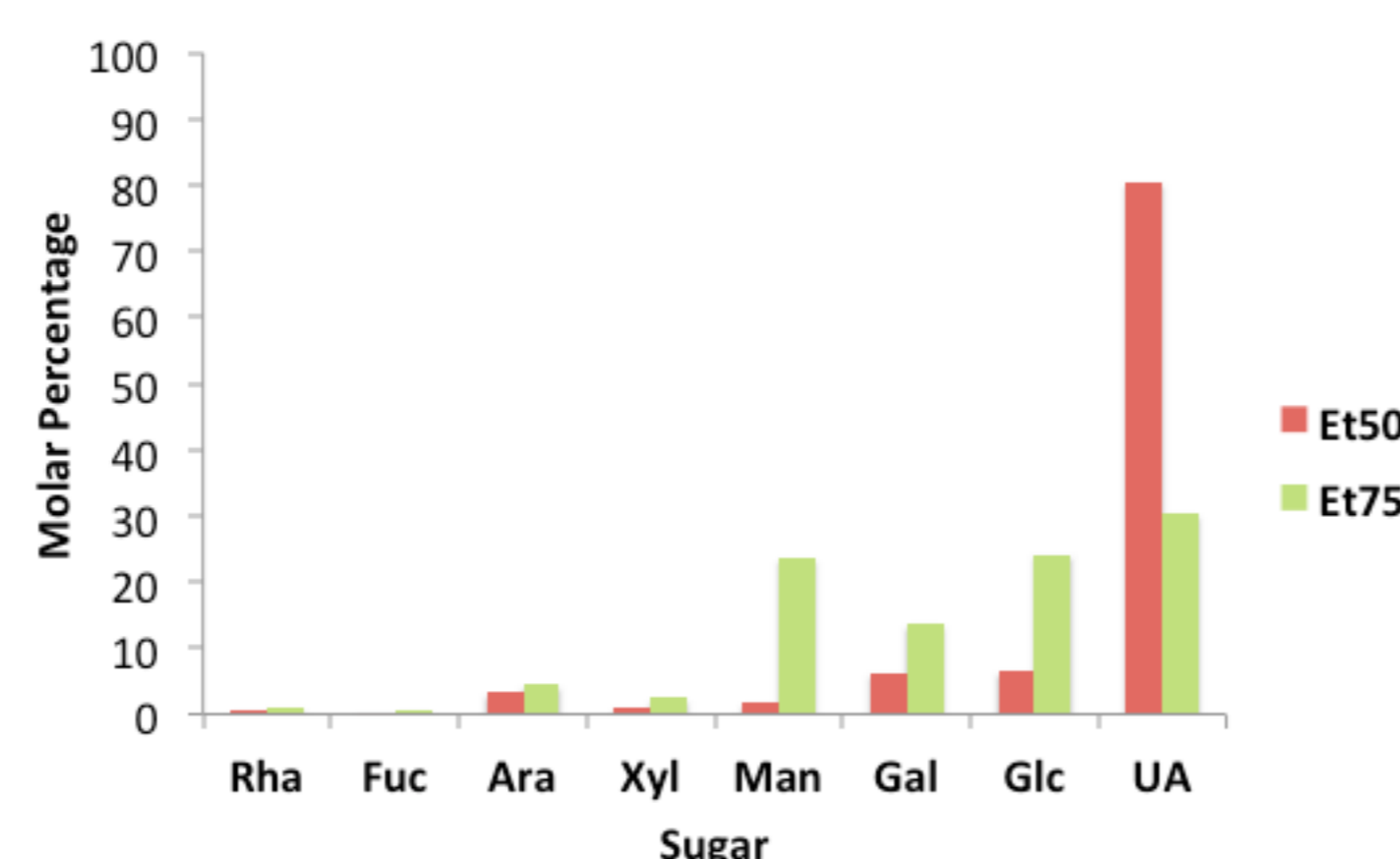


Figure 1- Polysaccharide content, expressed as mass percentage of the total polysaccharides recovered in the various fractions obtained through ethanol precipitation of the HMWM, and sugar composition of the richest fractions (Rha-rhamnose; Fuc-fucose; Ara-arabinose; Xyl-xylose; Man-mannose; Gal-galactose;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

- Most of the polysaccharides present in the HMWM precipitated in 50% and 75% aqueous ethanol solutions;
- The polysaccharides that precipitated in 50% aqueous ethanol solutions exhibited a monomeric composition particularly rich in UA residues, suggesting the presence of high proportions of pectic polysaccharides;
- The polysaccharides that precipitated in 75% aqueous ethanol solutions contained similar proportions of mannose, glucose and uronic acid residues, evidencing the presence of neutral polysaccharides mixed with pectic polysaccharides.

References

[1] A. M. P. Carvalho, Etnobotánica del Parque Natural de Montesinho. Plantas, tradición y saber popular en un territorio del nordeste de Portugal, 2005, PhD Thesis-Universidad Autónoma de Madrid.
[2] I.A. Schepetkin, M.T. Quinn, *International Immunopharmacology*, 2006, 6, 317-333.

Results

2. Anion-exchange chromatography (AEC)

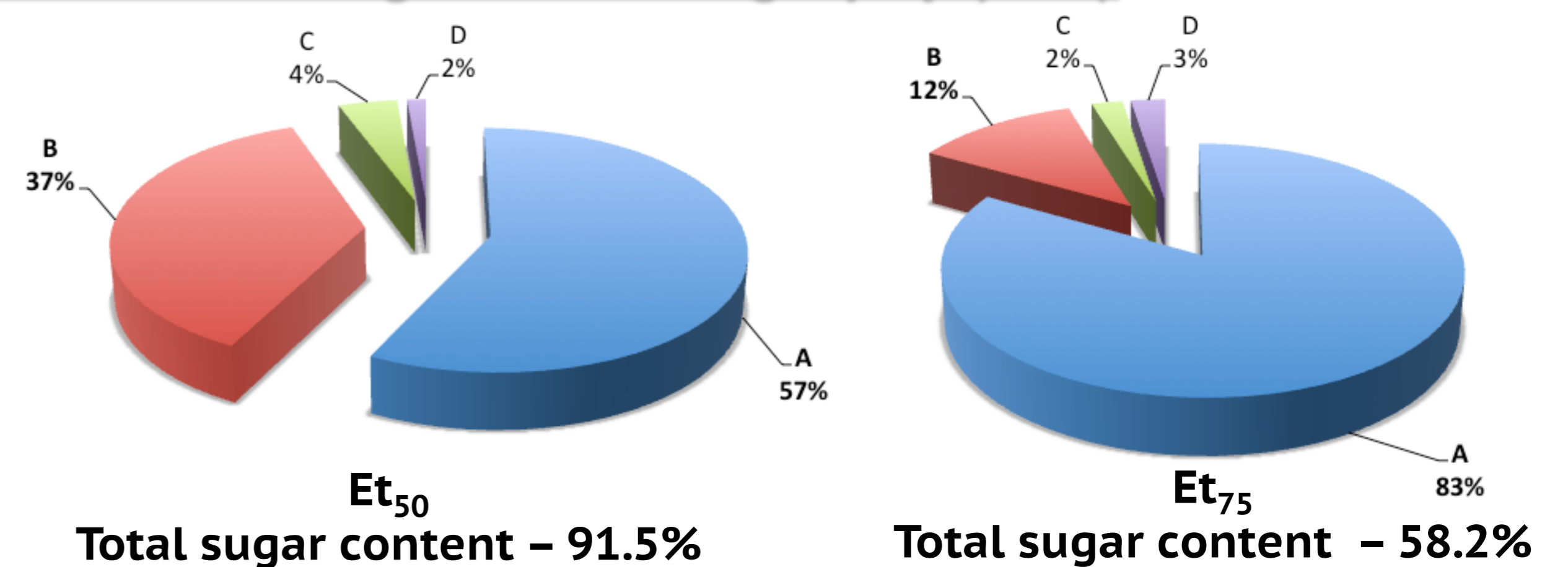


Figure 2- Polysaccharide content of the various fractions obtained through AEC of the Et₅₀ and Et₇₅ fractions, expressed as mass percentage of the total polysaccharides recovered in the various fractions.

Linkage	Molar %	Linkage	Molar %
2,4-Rhap	0.2	T-Manp	1.5
Total	0.2^a (0.0)^b	4-Manp	27.7
T-Fucp	0.2	2,4-Manp	1.9
Total	0.2 (0.0)	3,4-Manp	3.5
T-Araf	1.5	4,6-Manp	9.5
2-Araf	0.3	Total	44.1 (44.5)
3-Araf	0.5	T-Galp	9.5
5-Araf	1.6	2-Galp	0.2
3,5-Araf	0.2	5-Galp	0.7
Total	4.9 (5.1)	4-Galp	5.0
T-Xylp	2.4	6-Galp	2.9
2-Xylp	1.0	3,6-Galp	1.4
4-Xylp	1.1	Total	19.7 (20.1)
2,4-Xylp	0.5	T-Glcp	1.3
Total	5.0 (4.6)	1,3-Glcp	0.4
		4-Glcp	18.1
		6-Glcp	0.3
		2,4-Glcp	1.8
		3,4-Glcp	0.6
		4,6-Glcp	3.4
		Total	31.0 (37.1)

^a linkage analysis;
^b sugar analysis.

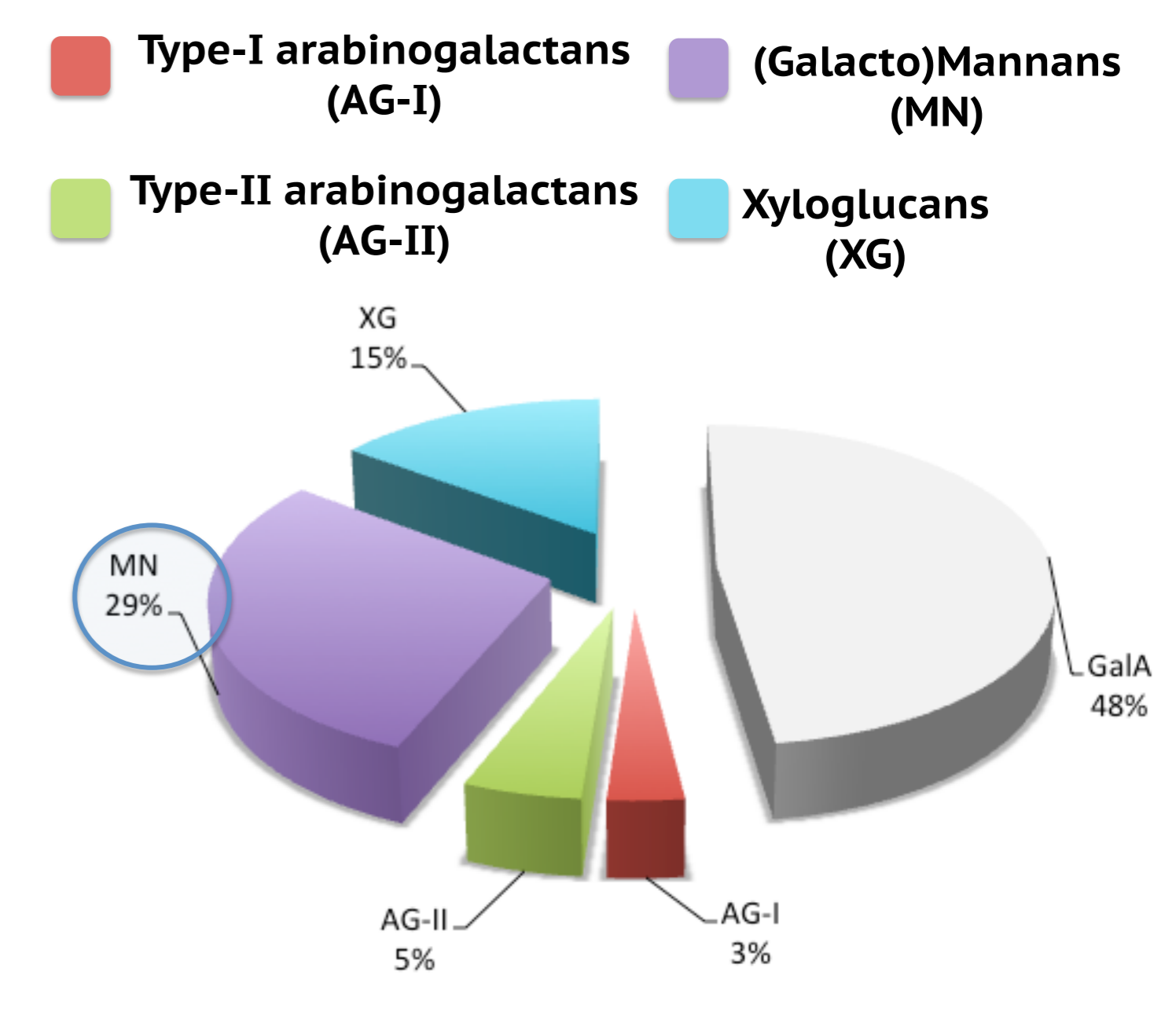


Figure 3- Deduced linkages for the Et₇₅A fraction, which exhibited the highest MN content (data not shown), and its polysaccharide composition, expressed as mass percentage.

3. ESI-MS and MS/MS

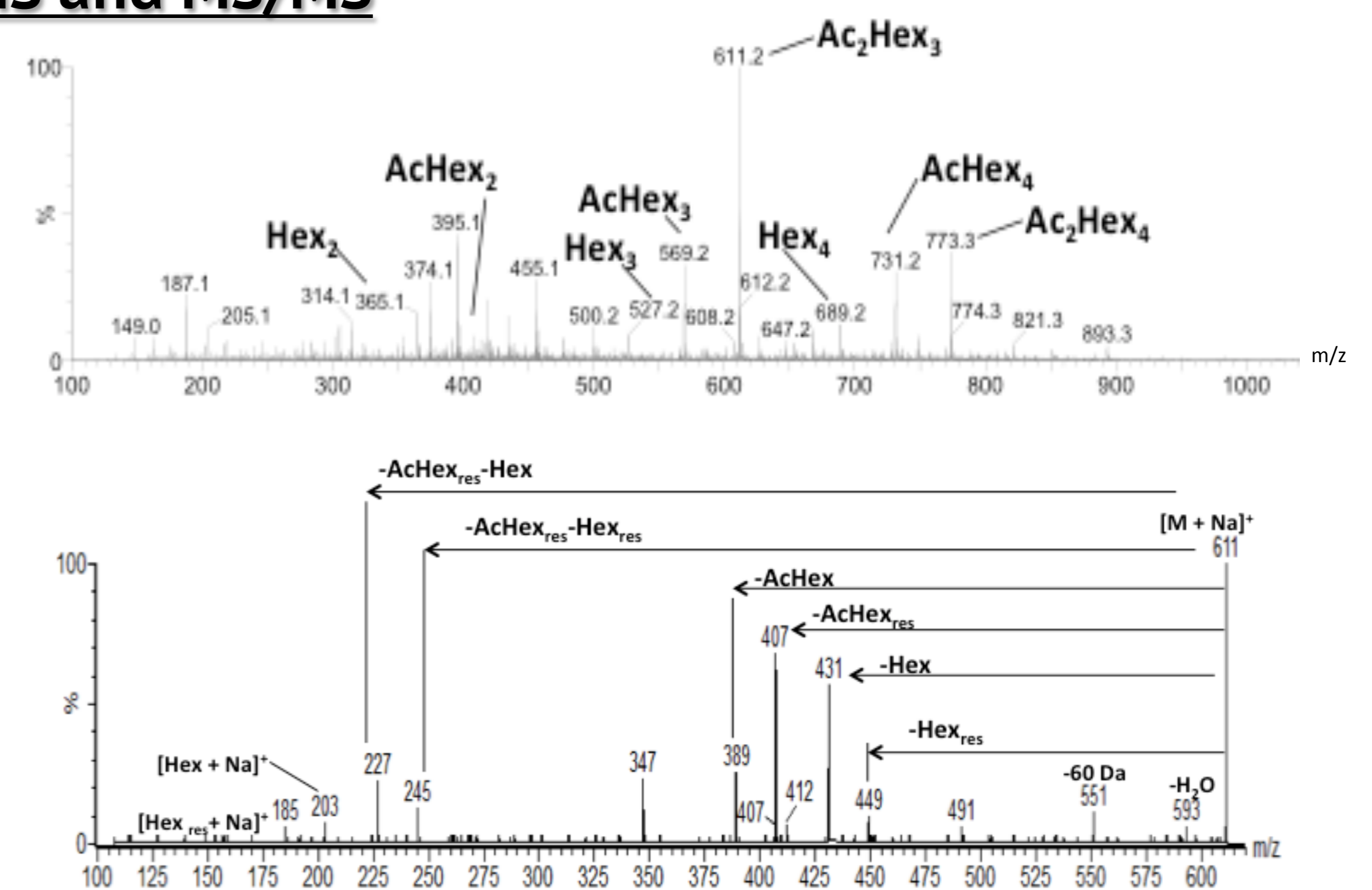


Figure 4- ESI-MS and MS/MS spectra obtained through *endo*-mannanase digestion of the Et₇₅A fraction, and subsequent SEC.

4. Macrophage immunostimulatory activity

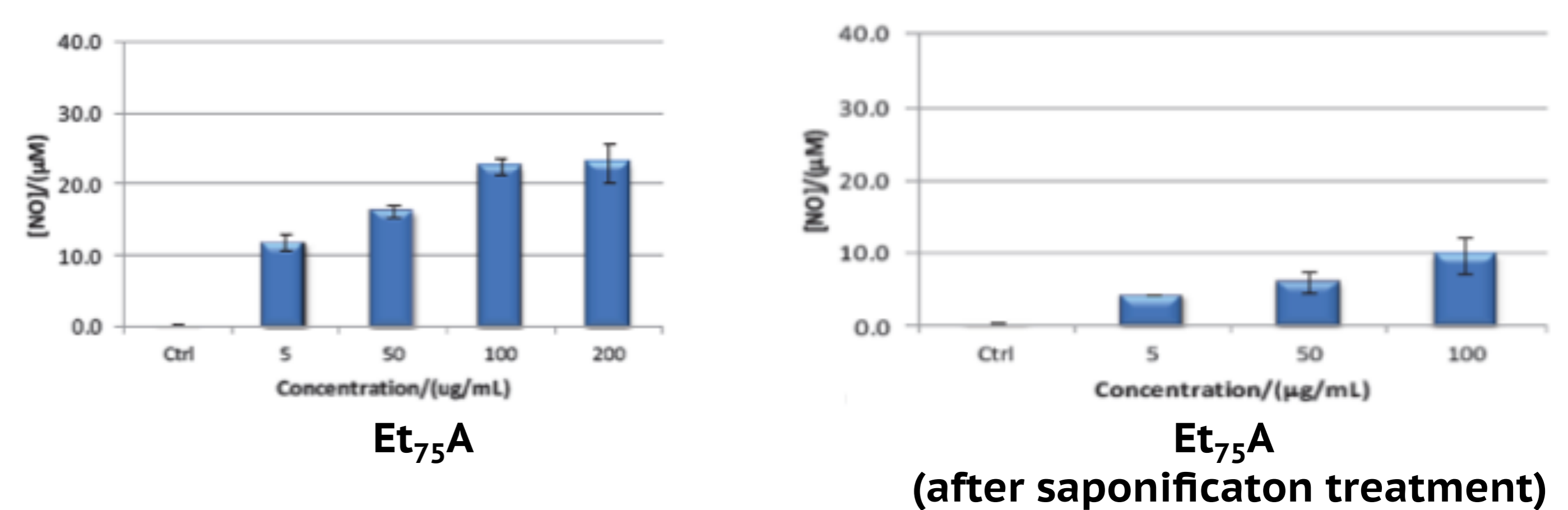


Figure 5- Macrophage immunostimulatory activity of the Et₇₅A fraction (before and after saponification treatment), expressed as NO production.

- The decrease in the macrophage immunostimulatory activity of the Et₇₅A fraction caused by the saponification treatment might be related with the de-acetylation of the galactomannans present in that fraction.

Conclusions

- The HWE from *P. tridentatum* dried inflorescences contain a mixture of polysaccharides, which evidenced macrophage immunostimulatory activity, and that comprise high proportion of pectic polysaccharides together with lower proportions of galactomannans, xyloglucans, and AG-II;
- The galactomannans acetylation seems to be play an important role in the observed macrophage immunostimulatory activity.