University of Aveiro
QOPNA

INSTITUTO POLITÉCNICO DE BRAGANÇA Escola Superior Agrária

CIMO Centro de Investigação de Montanha

CNC

Pectic polysaccharides from the infusions of *P. tridentatum, F. angustifolia and M. suaveolens*: structural characterization and modulation of the inflammatory activity

Vitor M. R. Martins [a,b] , Isabel V. Ferreira [c,d], Maria T. Cruz [c], Maria T. Batista[d] and Manuel A. Coimbra[b]

[a] CIMO, School of Agriculture, Polytechnic Institute of Bragança, 5301-855 Bragança, Portugal; [b] QOPNA, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal;
[c] CNC and Faculty of Pharmacy, University of Coimbra, 3004-517 Coimbra, Portugal ; [d] CEF, Faculty of Pharmacy, University of Coimbra, 3000-548 Coimbra, Portugal
E-mail address: vmartins@ipb.pt

Introduction

In Trás-os-Montes region (Portugal), the small shrub (*Pterospartum tridentatum*), the narrow-leafed ash (*Fraxinus angustifolia*), and the apple mint (*Mentha suaveolens*) are vegetable species used in the preparation of infusions for medicinal purposes, such as protection against diabetes, hypertension, high levels of cholesterol and uric acid [1]. These infusions contain several types of polysaccharides, such as pectic polysaccharides and galactomannans (GM´s), often reported as immunomodulators [2, 3]. Pectic polysaccharides are structurally complex polymers, exhibiting different polymeric building blocks: homogalacturonans (HG), type I rhamnogalacturonans (RG-I), type II rhamnogalacturonans (RG-II) and xylogalacturonans (XG) [4]. The backbone of RG-I can be partly substituted with, among others, type-II arabinogalactans (AG-I) that form ramified regions responsible for the modulation of the immune response [2]. For GM´s, factors like chain length, degree of branching and degree of acetylation seem to influence their immunomodulating activity [3].

Objectives

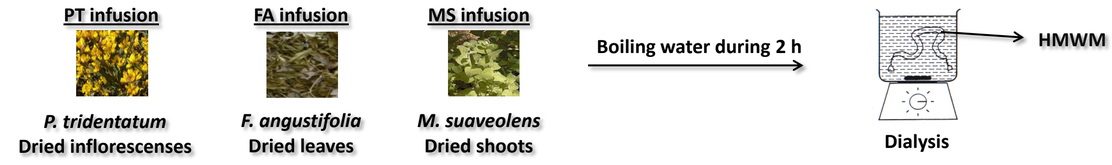
❖To extract and fractionate the polysaccharides present in the infusions obtained from the dried leaves of *F. angustifolia,* from the dried shoots of *M. suaveolens* and from the dried flowers of *P. tridentatum;*

❖To determine the monomeric composition and linkage composition of the polysaccharides present in the fractions obtained;

❖To evaluate the influence of the polysaccharides present in some of the obtained fractions in the inflammatory activity of macrophages.

Methods

❖**Infusions and High Molecular Weight Material (HMWM) Preparation**

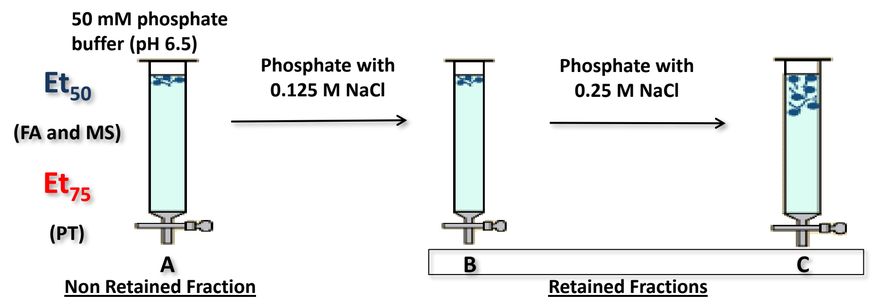


❖**HMWM Ethanol Fractionation Procedure**

HMWM (PT, FA and MS) → Ethanol Fractionation → Et_{50} - material insoluble in 50% ethanol; Et_{75} - material insoluble in 75% ethanol

All the obtained fractions were submitted to sugar analysis.

❖**Anion Exchange Chromatography (AEC) Fractionation Procedure**



All the obtained fractions were submitted to sugar and methylation analysis.

Results

❖**Monomeric composition of the fractions obtained by ethanol precipitation and anion exchange chromatography (AEC) of the HMWM**

Table I- Yield, total sugar content and monomeric composition of the fractions obtained by ethanol fractionation of the HMWM .

	Yield (%)	Total Sugars (mass%)	Monosaccharide Composition (mol %)						
			Rha	Ara	Xyl	Man	Gal	Glc	UA
P. Tridentatum		44.1	0.9	4.7	1.3	7.2	7.5	32.7	46.0
Et_{50}	43.9	74.3	0.5	3.2	1.0	1.8	6.2	6.6	80.7
Et_{75}	30.5	58.2	0.5	3.7	2.0	21.4	10.2	20.4	41.8
F. Angustifolia		57.0	4.2	7.6	1.6	2.7	5.3	11.3	67.4
Et_{50}	48.6	81.0	2.0	3.5	1.6	0.4	3.2	3.0	86.3
Et_{75}	16.9	55.8	3.4	11.1	2.6	2.6	12.7	9.8	57.8
M. Suaveolens		46.4	1.8	5.3	0.9	3.0	8.6	9.4	71.1
Et_{50}	39.5	72.0	1.3	3.0	0.4	0.4	3.4	2.0	89.5
Et_{75}	17.4	62.9	1.5	6.5	1.2	3.9	9.7	6.5	70.8

Et_{50}
❖Pectic polysaccharides "enriched" in HG domains

Et_{75}
❖Pectic polysaccharides "enriched" in RG-I domains.

Table II- Yield, total sugar content and monomeric composition of the major fractions obtained by AEC.

	Yield (%)	Total Sugars (mass%)	Monosaccharide Composition (mol %)						
			Rha	Ara	Xyl	Man	Gal	Glc	UA
P. Tridentatum Et_{75}_A	78.6	85.3	0.0	3.0	2.6	26.1	11.8	15.1	41.4
F. Angustifolia Et_{50}_C	72.7	86.3	1.8	5.1	1.9	0.0	4.2	0.4	86.6
M. Suaveolens Et_{50}_B	52.0	96.5	0.9	2.2	0.2	0.1	1.8	0.5	94.3
P. Tridentatum Et_{75}_A-I	---	86.7	0.2	4.5	4.3	46.9	16.4	25.6	2.2

❖ Pectic polysaccharides "enriched" in RG-I domains and relevant proportion of GM´s.

❖Pectic polysaccharides "enriched" in HG domains

❖**Inflammatory Evaluation Assays**

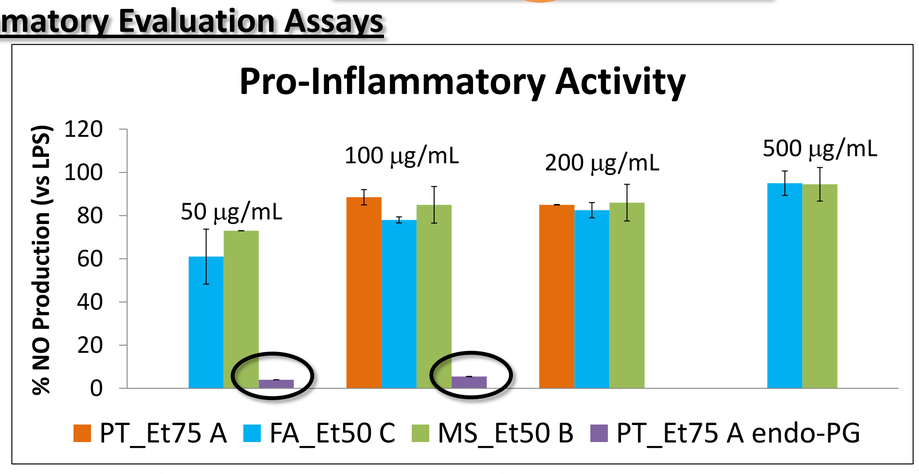
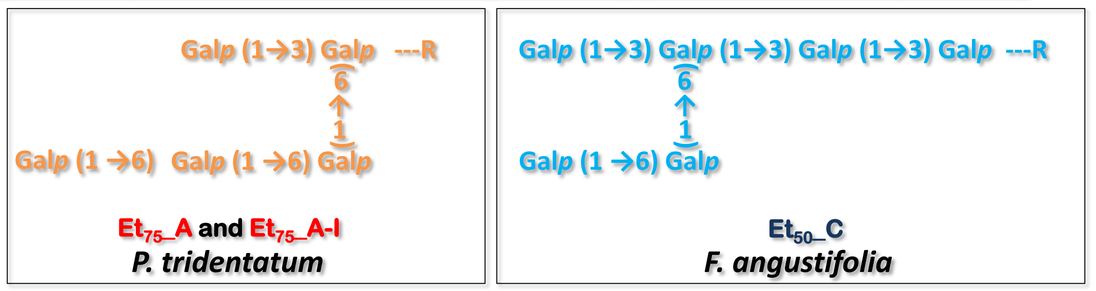
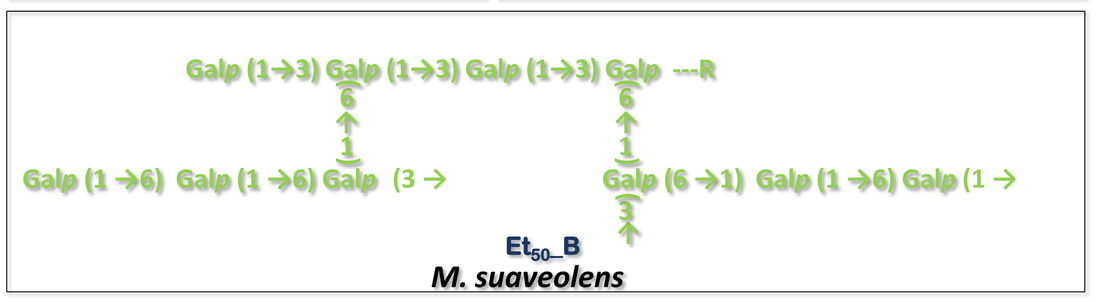


Figure 1- NO production determined by Griess reagent assay (average results from two independent experiments).

❖ The pectic polysaccharides from the 3 species assayed exhibited strong pro-inflammatory activity.
❖ When the fraction PT_Et75A was saponified and submitted to an *endo*-polygalacturonase digestion, a significant decrease in the NO production was observed (PT_Et75A *endo-PG)*.
❖ No anti-inflammatory activity or decrease of the cellular viability for the assayed concentrations were observed (data not shown).

❖**Proposed structures, based on methylation analysis, of the galactan moieties present in the fractions exhibiting pro-inflammatory activity**





❖ The proposed structures are similar to those reported and considered to contribute for the immune response activation exhibited by diverse medicinal plants [2].
❖ The loss of activity of the fraction treated with endo-PG showed that the presence of galacturonic acid seems to be important for the modulation of the inflammatory activity.

Conclusion

❖ The infusions from *P. tridentatum*, *F. angustifolia* and *M. suaveolens* contain pectic polysaccharides with ramified galactan domains similar to those reported to contribute for the immune response activation.
❖ The infusions of *P. tridentatum* also contain GM´s reported for their immunostimulatory activity.
❖ The loss of pro-inflammatory activity observed after saponification and *endo*-PG digestion , with retention of the galactan and GM moiety, shows that other factors beyond these polysaccharides are involved in the activity.
❖ It is possible that the acetylation of the GM´s, loss during the saponification, could play an important role in the immunostimulatory activity of these polysaccharides [3]. Also, it is possible that a certain proportion of galacturonic acid residues (or esterified moieties), removed by the *endo*-PG treatment, could be determinant for the activity of these polysaccharides.
❖ Further studies are needed to clarify the real contribution of the degree of acetylation of the GM´s and of the galacturonic acid residues of these 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] M.H. Sakurai, H. Kiyohara, T. Matsumoto, Y. Tsumuraya, Y. Hashimoto, H. Yamada, *Carbohydrate Research*, 1998, **311**, 219-229.
[3] J. Simões, F.M. Nunes, P. Domingues, M.A. Coimbra, M.R. Domingues, *Carbohydrate Polymers*, 2012, **90,** 229-236.
[4] S. Perez, M.A. Rodriguez-Carvajal, T. Doco, *Biochimie,* 2003, **85**, 109-121.

Thanks are due to Fundação para a Ciência e a Tecnologia (FCT, Portugal), European Union, QREN, FEDER, and COMPETE for funding the QOPNA (project PEst-C/QUI/UI0062/2013; FCOMP-01-0124-FEDER-037296) and CIMO research units.

FCT Fundação para a Ciência e a Tecnologia · COMPETE · QREN Quadro de Referência Estratégico Nacional · União Europeia Fundo Europeu de Desenvolvimento Regional · Governo da República Portuguesa