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POLYSACCHARIDES FROM THE INFUSIONS OF *P. TRIDENTATUM*, *F. ANGUSTIFOLIA*, AND *M. SUAVEOLENS*

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Introduction

The use of tea for medicinal purposes is a rather ancient and old practice, whose chemical aspects are well known [1]. However, in folk medicine there is a great diversity of plants that are used as infusions for such purposes. Although the biological activity and potential health benefits of some of these infusions are well documented, as is the case of chamomile [2], most knowledge about the infusions is based on folk tradition passed over several generations, without a sound scientific basis.
In Portugal, in Trás-os-Montes region, the small shrub (*Pterospartum tridentatum)*, the narrow-leafed ash (*Fraxinus angustifolia*) and the apple mint (*Mentha suaveolens*) are plants used for medicinal purposes [3]. According to the popular tradition, the infusions of *P. tridentatum* inflorescences protect against cold, headache, stomachache, throat irritation, diabetes, high blood pressure, urinary tract diseases, and heart problems; the infusions of *F. angustifolia* dried leaves protect against high levels of cholesterol, blood pressure, and uric acid, and act against rheumatism; the infusions of *M. suaveolens* shoots have benefits for stomach, diarrhoea, cold, are anti-haemorrhagic and anti-cholesterolemic [3]. These health benefits are associated with the presence of active compounds such as phenolics, essential oils, and polysaccharides. Although phenolics and essential oils have been studied in detail for more than two decades, the structures of the polysaccharides present in plant infusions and their involvement in the health benefits is still incipient. Therefore, in this work, a first approach on the structure of the polysaccharides present in the infusions of *P. tridentatum* dried inflorescences, *F. angustifolia* dried leaves, and *M. suaveolens* dried shoots is presented.

Materials and Methods

Infusions and preparation of polymeric fractions

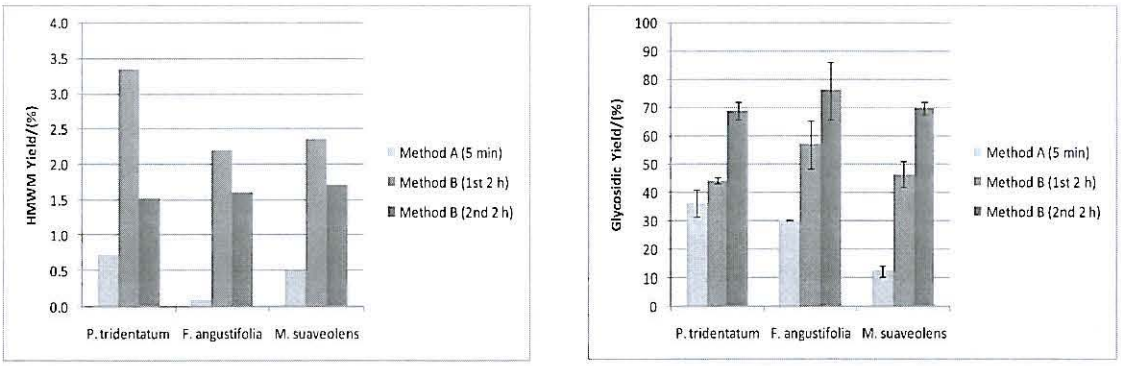
The infusions of *P. tridentatum* dried inflorescences, *F. angustifolia* dried leaves, and *M. suaveolens* dried shoots (100 g/L) were prepared using two distinct methods, using different times of hot water extraction: 5 min in Method A and Method B, 4 h, divided in two periods of 2 h. The obtained infusions were filtered, dialyzed (12-14 kDa) and freeze-dried to obtain the high molecular weight material (HMWM).
The HMWM obtained from Method B was graded precipitated in ethanol [4] to give the material that precipitated in 50% ethanol (Et_{50}), 75% ethanol (Et_{75}), and the supernatant solution (SN).

Sugar and linkage analysis

Neutral sugars were released by acid hydrolysis and were analyzed as their alditol acetates by GC-FID [5]. Uronic acids were determined colorimetrically by 3-phenylphenol [6]. Polysaccharides were methylated with methyl iodide, hydrolyzed, derivatized partially methylated alditol acetates and analyzed by GC-MS [7].

Results and Discussion

In order to study the structural features of the polysaccharides present in the infusions of *P. tridentatum* dried inflorescences, *F. angustifolia* dried leaves and *M. suaveolens* dried shoots it was necessary to obtain HMWM in considerable quantities and sufficiently rich in glycosidic material.



a) **b)**

Figure 1. **a)** HMWM and **b)** glycosidic yields for the infusions of *P. tridentatum*, *F. angustifolia* and *M. suaveolens* obtained by Method A (15 min) and Method B (1st 2 h and 2nd 2 h).

The infusions prepared by Method B (1st and 2nd 2h of the infusion process) exhibited a HMWM yield higher than those prepared by Method A (Fig. 1a), showing that the higher period of infusion (5 min *vs* 4 h) is responsible for the increases of 7 times, 19 times and 8 times in the HMWM yield in *P. tridentatum*, *F. angustifolia*, and *M. suaveolens*, respectively. Although the amount of material extracted was higher in the 1st 2 h of the infusion process, the amount of material extracted in the following 2 h was still relevant. This observation suggests that even though the extraction rate diminished throughout the infusion process, which is in agreement with the usual kinetics of the extraction processes, still a large amount of material can be extracted when the material is again suspended in boiling water for an additional 2 h. This allows extracting more 45% of the material extracted in the first 2 h for *P. tridentatum*, and 73% in *F. angustifolia* and *M. suaveolens*. Generally, regardless of the method, infusions of *P. tridentatum* showed higher yields of HMWM, as dried inflorescences were used in the preparation of *P. tridentatum* infusions, while the leaves and shoots were used in *F. angustifolia* and *M. suaveolens* infusions, respectively.

Fig. 1b shows that the HMWM obtained from infusions prepared by Method B were richer in polysaccharides, particularly those from F. angustifolia and M. suaveolens. It was also found that generally the HMWM obtained in the 2nd 2 h of the infusion process was richer in polysaccharides, suggesting a possible preferential extraction of polysaccharides during that period, while in the 1st 2 h of the infusion process might occur a more significant co-extraction of other types of compounds, such as phenolics. Therefore, based on these results, and taking in account that the main subject of this study are polysaccharides, Method B was elected as the most suitable for this purpose and the monomeric composition of the HMWM obtained was analysed.

For all samples, the HMWM were rich in uronic acids (UA) besides having significant proportions of Ara, Gal, and Glc (Table I). This sugars composition suggests the presence of pectic polysaccharides. The HMWM obtained from the 1st 2 h of infusions was considerably richer in Glc than those from the 2nd 2 h. As Glc is not usually referred as a component of pectic polysaccharides, it can be inferred that other compounds are also extracted alongside with these polysaccharides. This assumption was supported by the darker colour, characteristic of the presence of phenolic compounds, of the HMWM obtained from the 1st 2 h of the infusion process.

In order to separate the glycosidic compounds present in the HMWM obtained from the infusion process (1st and 2nd 2 h), a fractionation by ethanol precipitation was performed, allowing obtaining three fractions with distinct ethanol solubility for each type of vegetable material: Et_{50}, material precipitated with 50% ethanol; Et_{75}, material precipitated with 75% ethanol; and SN, material that remained soluble in 75% ethanol solution.

Table I. Total sugar content and monosaccharide composition of the HMWM from the infusions of *P. tridentatum* dried inflorescenses, *F. angustifolia* dried leaves, and *M. suaveolens* dried shoots.

	Yield (%)	Total Sugars (mass%)	Monosaccharide Composition (mol %) Rha	Ara	Xyl	Man	Gal	Glc	UA
P. tridentatum 1st 2 h		44.1	0.9	4.7	1.3	7.2	7.5	32.7	46.0
Et_{50}	43.9	91.3	0.5	3.3	1.1	1.8	6.2	6.6	80.7
Et_{75}	30.5	69.5	0.6	3.7	2.0	21.4	10.2	20.4	41.8
SN	25.6	24.7	1.0	8.9	1.4	4.0	4.3	65.9	14.7
F. angustifolia 1st 2 h		57.0	1.8	5.3	0.9	3.0	8.6	9.4	71.1
Et_{50}	48.6	81.0	2.1	3.5	1.6	0.5	3.2	3.0	86.3
Et_{75}	16.9	55.8	3.4	11.2	2.6	2.6	12.8	9.8	57.8
SN	34.5	33.0	8.4	17.0	1.2	11.0	5.3	33.9	23.3
M. suaveolens 1st 2 h		46.4	4.2	7.6	1.6	2.7	5.3	11.3	67.4
Et_{50}	39.5	97.4	1.3	3.0	0.5	0.5	3.5	2.0	89.5
Et_{75}	17.4	63.8	1.5	6.5	1.3	3.9	9.7	6.5	70.8
SN	43.1	26.2	3.2	5.0	1.5	8.5	16.9	23.6	41.5
P. tridentatum 2nd 2 h		68.8	1.0	10.8	1.0	4.0	8.6	11.4	63.2
Et_{50}	31.1	99.5	0.8	6.5	1.0	2.0	8.3	3.7	78.0
Et_{75}	36.1	94.5	0.7	6.6	1.1	6.4	9.5	6.0	69.7
SN	32.8	37.5	1.3	33.2	1.3	4.7	4.7	30.5	24.4
F. angustifolia 2nd 2 h		76.1	1.6	6.8	0.8	1.7	8.1	4.8	76.4
Et_{50}	49.6	90.1	1.6	4.3	1.5	0.2	2.9	1.1	88.5
Et_{75}	24.1	84.7	2.5	10.6	2.6	1.1	9.5	4.1	69.8
SN	26.3	38.8	5.3	35.7	1.2	8.0	4.5	22.3	23.1
M. suaveolens 2nd 2 h		69.7	2.5	11.6	1.8	1.5	5.6	5.3	71.8
Et_{50}	59.9	91.1	1.2	3.8	0.4	0.2	3.8	1.0	89.7
Et_{75}	12.6	63.9	1.7	8.3	1.2	3.3	11.4	4.7	69.6
SN	27.5	29.4	2.1	9.6	1.7	7.6	22.2	19.5	37.4

Et_{50} fractions were particularly rich in glycosidic material (81.0-99.5%), with more than 75% of uronic acids (UA). Although in small proportions, neutral sugars such as Ara (mostly terminally- and 5-linked, with small proportions of 3- and 3,5-linked residues) and Gal (mostly terminally- and 6-linked, although 3- and 3,6-linked residues were also

present) were detected, suggesting the presence of Type II arabinogalactans. Rha (terminally-, 2-, and 2,4-linked) residues were also detected. This and the presence of high proportions of UA, may indicate the possible presence of rhamnogalacturonan chains, which may be carrying Type II arabinogalactan chains. Xyl and Glc residues were detected in all plant infusions, suggesting the presence of other polysaccharides.
Et_{75} fractions were rich in glycosidic material (55.8-95.5%), although of less acidic nature than the Et_{50} fractions, reflected by the presence of lower amounts of UA when compared with the Et_{50} fractions. For all plant infusions, the Et_{75} fractions exhibited relevant proportions of Ara, Gal, and Glc, which, although with different proportions, featured the same type of linkages detected in the Et_{50} fractions, indicating the presence of the same type of polysaccharides. Et_{75} fraction obtained from the 1st 2 h of *P. tridentatum* infusion also exhibited glycosidic material rich in Man (21.4%), mostly terminally- and 4-linked, indicating the possible presence of mannans. Although in small proportions, Rha and Xyl residues were detected in all plant infusions.
SN fractions were relatively poor in sugars, with the ones obtained in the 2nd 2 h of the infusion process presenting slightly higher values: 37.5%, 38.8%, and 29.4%, for *P. tridentatum*, *F. angustifolia*, and *M. suaveolens*, respectively. These fractions were poorer in glycosidic material, particularly UA, than Et_{75} and Et_{50} fractions, indicating that the more acidic glycosidic material precipitates first, being collected in the Et_{50} fractions, while the less acidic one remains soluble after a 75% ethanol addition, being collect in the SN factions. The *P. tridentatum* and *F. angustifolia* infusions exhibited relevant proportions of Ara (mostly terminally-, 5-, and 3,5-linked residues), Glc (mostly terminally-, 4-, and 6-linked) and UA, while the infusions of *M. suaveolens* featured relevant proportions of Gal (mostly terminally- and 6-linked), Glc (mostly terminally-, 4-, and 6-linked), and UA.

Conclusions

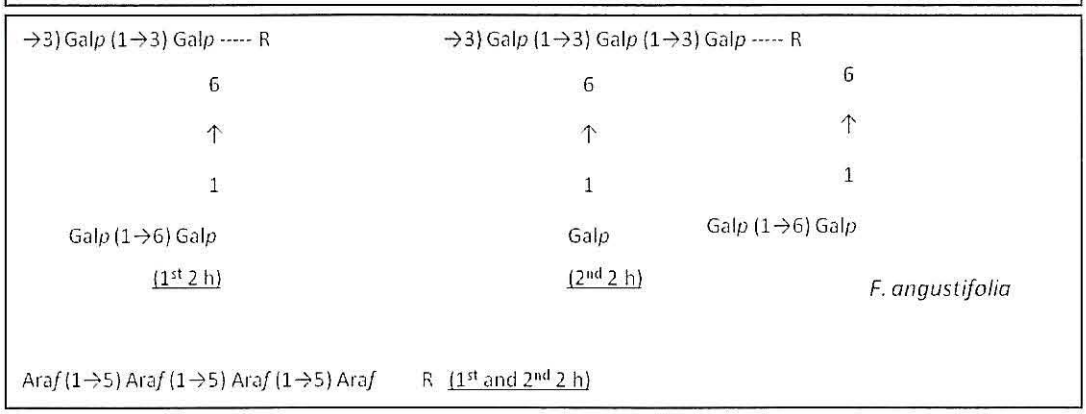
The treatment with boiling water with two steps of 2 h allowed to obtain higher yields of polysaccharides, particularly obtained in the 2nd of 2 h. These are pectic polysaccharides, namely, rhamnogalacturonan and type II arabinogalactans with distinct length and degree of ramification. Based on sugars and linkage analysis, the tentative structural features of the arabinan and galactan moieties for *P. tridentatum* and *F. angustifolia* are the following:

Gal*p* (1→6) Gal*p* (1→6) Gal*p* (1→6) Gal*p* -----R (1st 2 h)

Gal*p* (1→6) Gal*p* (1→6) Gal*p* -----R (2nd 2 h)

Ara*f* (1→5) Ara*f* (1→5) Ara*f* -----R (1st and 2nd 2 h)

P. tridentatum



F. angustifolia

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