angustifolia Leave Infusions

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Introduction

The use of plants with medicinal purposes is an ancient practice, which is still very common all around the world and that has been largely increasing over the last decades. Data from the World Health Organization indicate that 80% of the total human population still treat their health problems with traditional remedies [1]. Consequently, a large number of ethnobotanical research studies focused on the flora of diverse countries can be found in the literature [1,2]. According to these ethnobotanical studies, the infusion is one of the most frequently cited preparation processes. The infusions of "freixo" (*Fraxinus angustifolia*) dried leaves protects against high levels of cholesterol, blood pressure, and uric acid, and act against rheumatism [3]. These health benefits are associated with the presence of several physiologically active substances, such as phenolic compounds, essential oils, and polysaccharides, among others [4]. Although phenolic compounds have been studied in detail for more than two decades, the characterization of the polysaccharides present in plant´s infusions and their involvement in the health benefits is still incipient. The aim of this work is to provide a first insight into the polysaccharide composition of the infusions from Fraxinus angustifolia dried leaves.


Fraxinus angustifolia tree.


Fraxinus angustifolia leaves.

Methodology

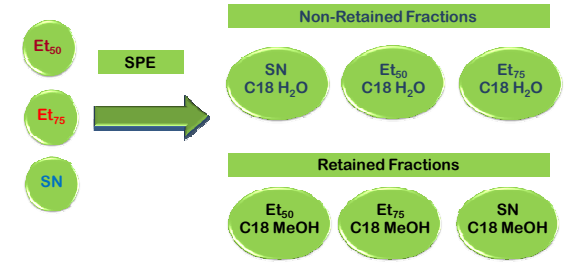
Infusion Time: 5 minutes


20 g dried leaves


150 mL boiling water

Dialysis → HMW Material 12-14 kDa Dial → Ethanol Precipitation → Et_{50}, Et_{75}, SN, WI

Scheme I- Infusion preparation, dialysis and ethanol precipitation of the high molecular weight (HMW) material.



Scheme II- Solid phase extraction (SPE) using C_{18} cartridges..

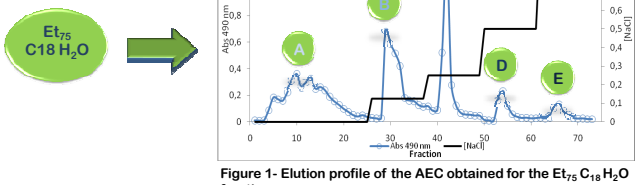


Figure 1- Elution profile of the AEC obtained for the Et_{75} C_{18} H_2O fraction.

Scheme III- Anion exchange chromatography (AEC) using DEAE-Sepharose .

Results

Table I- Sugars, phenolic content and yield of the high molecular weight fraction (Dial) and fractions obtained by ethanol precipitation (Et_{50}, Et_{75}, SN and WI).

Fraction	Yield (% w)	Total Sugars (% w)	Phenolics (% w)	UA (% mol)
Dial	2[a]	27	9	19
Et_{50}	18[b]	21	9	24
Et_{75}	21[b]	32	8	16
SN	41[b]	27	12	11
WI	20[b]	10	nd	27

[a] yield in relation to the total soluble material from infusions
[b] yields in relation to the total HMW material submitted to ethanol precipitation
nd non determined

•With the exception of WI fraction, the ethanol precipitation of the HMW material originated fractions with similar percentages of sugars, phenolics and monomeric composition.

•Analysis of the glycosidic linkage composition (data not shown) evidenced the presence of (1→6)-, (1→3,6)- and (1→3)-Gal with a proportion of 5:3:1, respectively, and also a high percentage of Ara terminally linked, diagnostic of the presence of Type II arabinogalactans.

•Dial fraction, accounting for 2% of the total soluble material obtained from the infusions, contained 27% of sugars, showing a monomeric composition rich in Ara, Gal, Glc and uronic acids.

Table II- Sugar composition and linkage analysis of the Dial fraction.

Linkage	Molar %
T-Rha*p*	7
2-Rha*p*	1
Total	**8[a] (8)[b]**
T-Fuc*p*	1
Total	**1 (1)**
T-Ara*f*	14
2-Ara*f*	1
3-Ara*f*	3
5-Ara*f*	5
Total	**23 (15)**
T-Xyl*p*	4
2-Xyl*p*	1
4-Xyl*p*	1
2,4-Xyl*p*	2
Total	**8 (3)**
2-Man*p*	1
4-Man*p*	5
4,6-Man*p*	1
Total	**7 (6)**
T-Gal	6
3-Gal*p*	2
6-Gal*p*	9
3,6-Gal*p*	6
Total	**23 (17)**
T-Glc*p*	14
4-Glc*p*	10
6-Glc*p*	7
Total	**31 (30)**

[a] data from linkage analysis
[b] data from sugar analysis

Table II- Sugar composition , phenolic content and yield of the fractions obtained by SPE.

Fraction	Yield[a] (% w)	Total Sugars (% w)	Phenolics (% w)	Monosaccharide Composition (mol %)							
				Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA
Et_{50} C_{18} H_2O	34	29	6	7	1	14	4	4	27	9	35
Et_{75} C_{18} H_2O	30	38	5	6	1	17	2	5	36	8	26
SN C_{18} H_2O	25	26	7	6	6	8	4	8	18	29	21
Et_{50} C_{18} MeOH	48	11	7	18	1	6	2	4	6	49	14
Et_{75} C_{18} MeOH	44	14	9	14	1	10	3	4	11	42	15
SN C_{18} MeOH	67	23	11	13	0	12	1	5	6	53	11

[a] yields in relation to the total material applied in the C_{18} cartridge

•SPE allowed to obtain lower yield non-retained fractions richer in sugars, with a monomeric composition rich in Rha, Glc and uronic acids..

•Retained fractions exhibited higher yields and quantities of phenolic compounds, while displaying lower sugar amounts and monomeric compositions richer in Rha and Glc.

Table III- Sugar composition and yield of the fractions obtained by AEC.

Fraction	Yield (% w)	Total Sugars (% w)	Monosaccharide Composition (mol %)							
			Rha	Fuc	Ara	Xyl	Man	Gal	Glc	UA
A	52.1	3	0	17	1	5	50	5	19
B	54.0	6	0	24	2	2	38	4	25
C	37.6	8	1	14	3	4	23	7	40
D	22.1	6	0	12	3	7	25	18	30
E	11.3	8	0	10	0	0	18	29	35

[a] yields in relation to the total material applied in the C_{18} cartridge

•AEC allowed the isolation of neutral and acidic fractions richer in sugars composed essentially of Ara, Gal and uronic acids.

•More acidic fractions, poorer in sugars, showed a monomeric composition rich in Ara, Gal, Glc and uronic acids.

Conclusions

•Infusions from the leaves of *Fraxinus angustifolia* contained high molecular weight material with 27% of sugars, comprising high percentages of Ara, Gal, Glc and uronic acids.

•Linkage analysis suggests the presence of Type II arabinogalactans. This type of polysaccharide is known for their anti-ulcer-, radical scavenging- and immunomodulating activities.

•The adopted procedure allowed obtaining fractions richer in sugars with a monomeric composition similar to the one from HMW material.

•Further studies need to be performed in the fractions of interest in order to fully elucidate the detailed structure of the polysaccharides present.

References

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