Tramesan, a Novel Polysaccharide from *Trametes versicolor*. Structural Characterization and Biological Effects.

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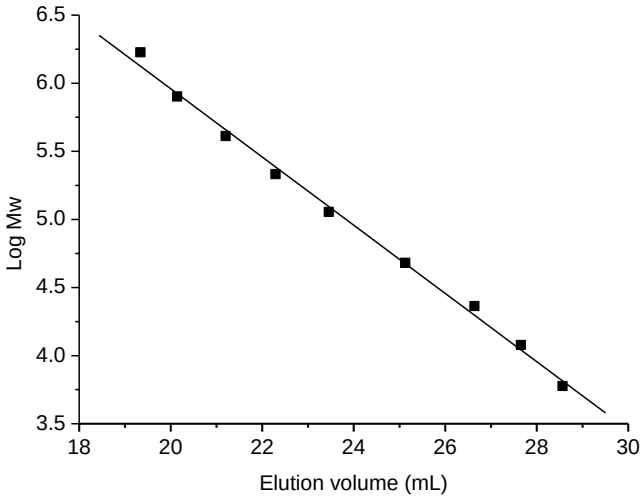

Pullulan molecular mass (kDa)
1660
788
404
212
112
47.3
22.8
11.8

Figure A. HP-SEC calibration plot obtained with nine pullulan standards with molecular masses in the range $1.7x10^6$ – $5.9x10^3$, used to evaluate Tramesan fraction molecular masses.

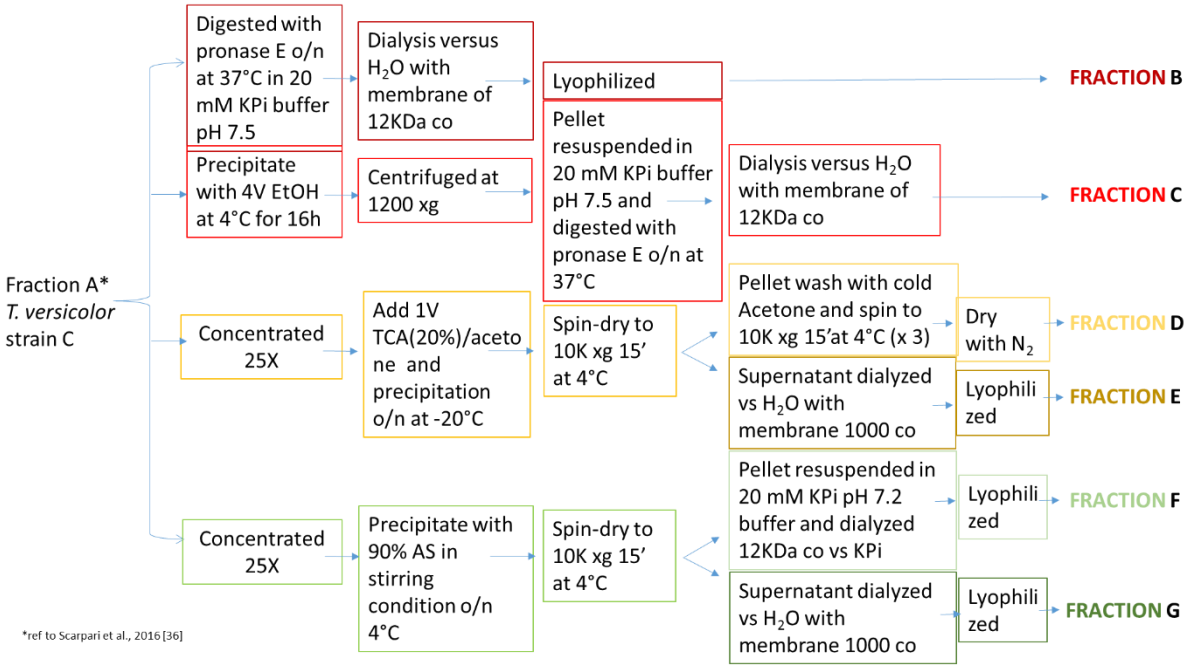

Figure B. Scheme summarizing the purification steps applied for the obtainment of the different fractions (B-G) used in the aflatoxin inhibition bioassays.

Table A. Composition analysis of the fraction C containing the polysaccharide produced by *T. versicolor* Tv117 (PLS 117) and of a *Trametes versicolor* commercial powder sample (CP). Results are expressed in weight %.

monosaccharide	Sample	
	PLS TV117	CP
Fuc	1.13	-
Man	19.72	48.10
Gal	17.18	1.41
Glc	61.98	50.49

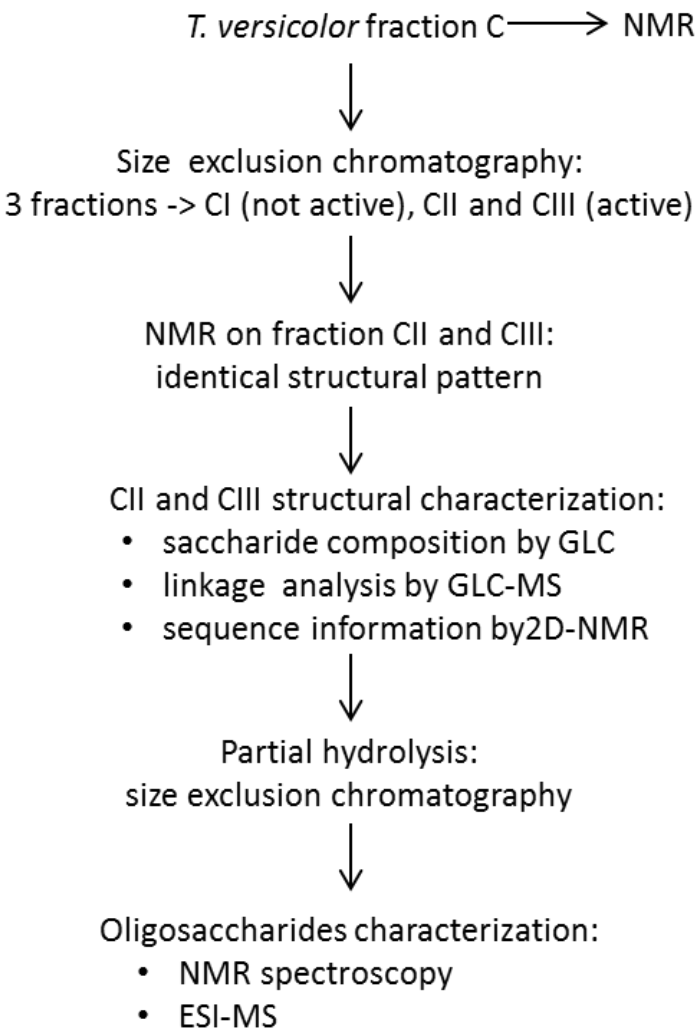

Figure C. Scheme of the characterization of the bioactive polysaccharide fraction C.

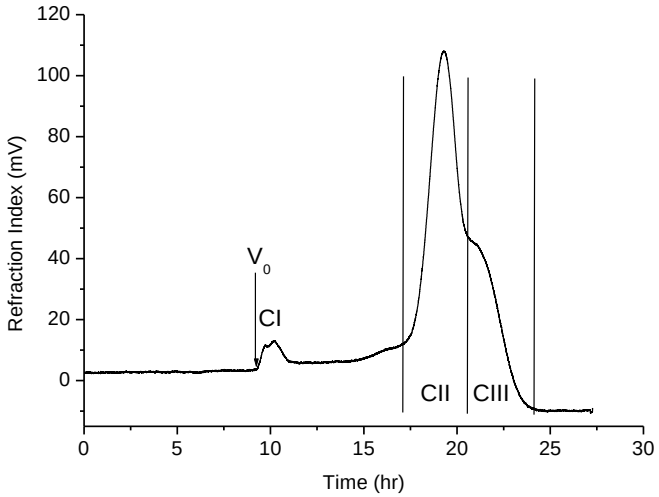

Figure D. Size exclusion chromatography on a Sephacryl S-300 column of the filtrate C from *T. versicolor* TV117 cultures. The three obtained fractions (CI, CII, CIII) are indicated.

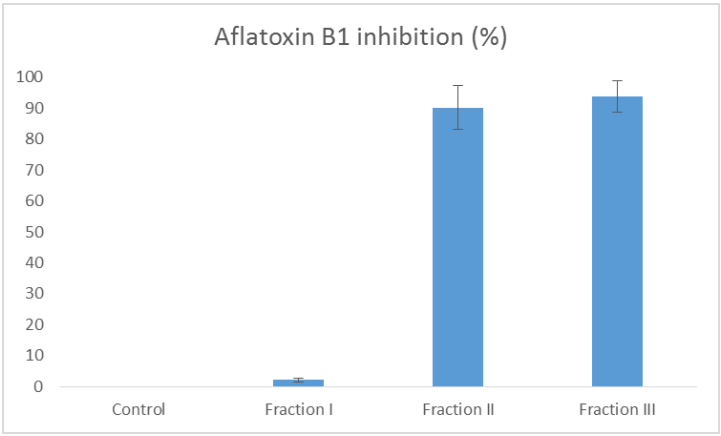

Figure E. Aflatoxin inhibition by the different SEC-separated fractions (I-III)

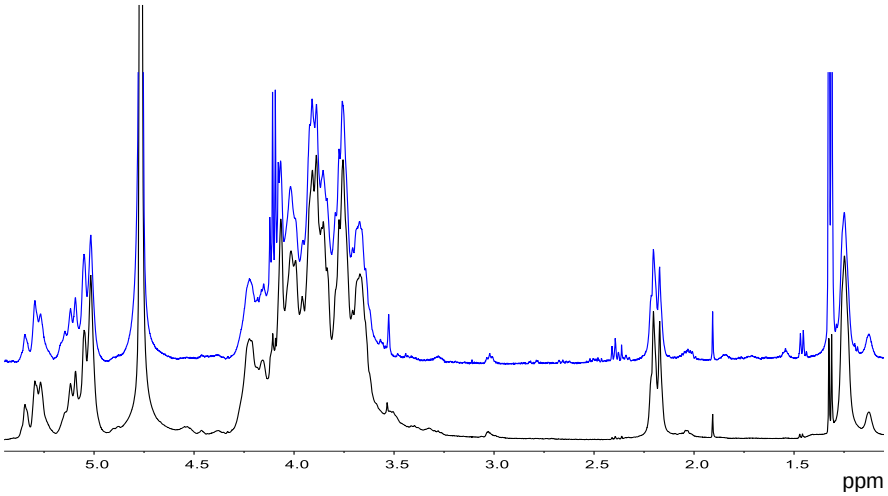

Figure F. ^{1}H-NMR spectra of the fractions II (red) e III (blue) obtained after size exclusion chromatography on a Sephacryl S-300 column.

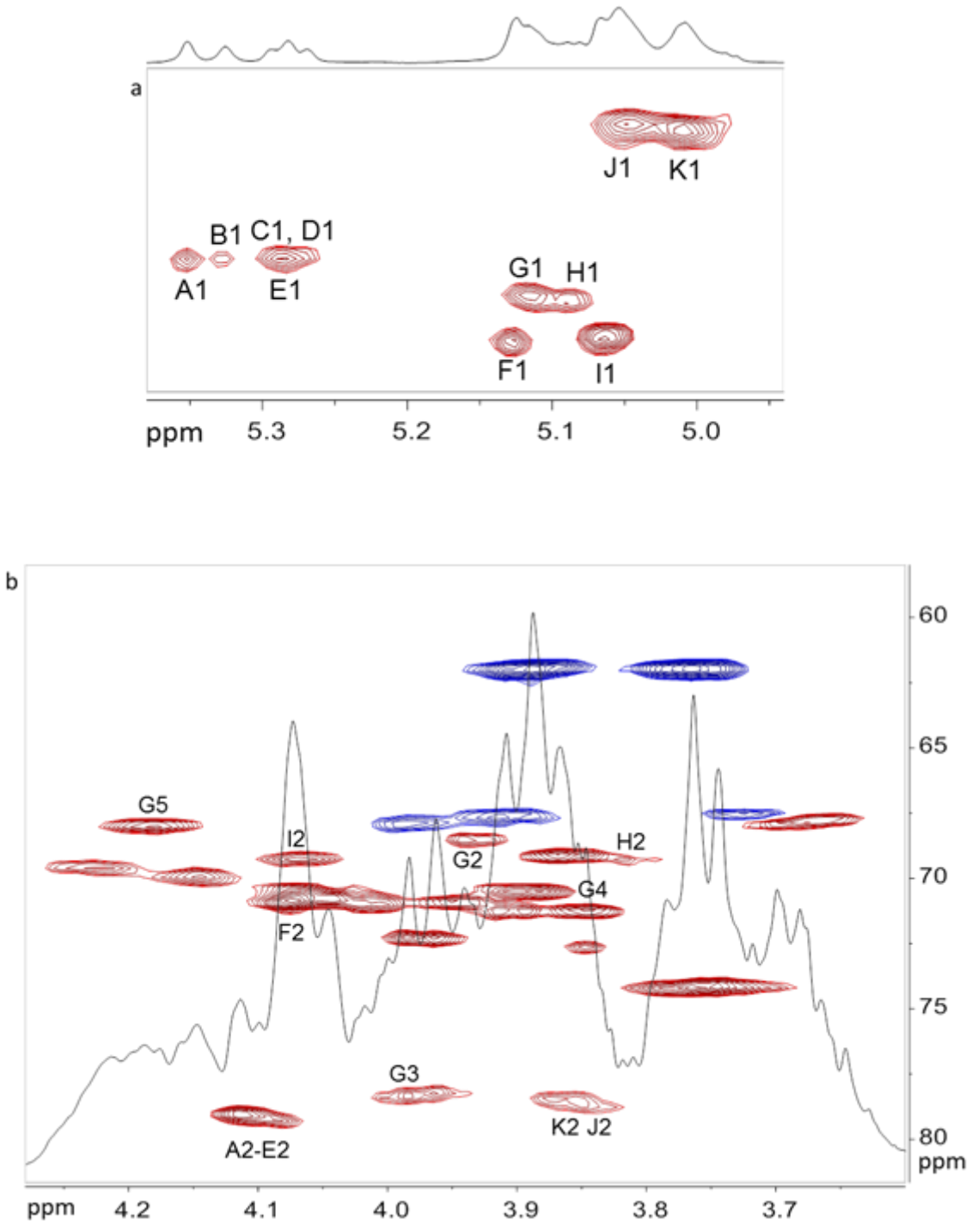

Figure G. HSQC plots of Trametan fraction III solution recorded at 50 °C. A) Expansion of the anomeric region and B) expansion of the ring region. Assignments as reported in **S2 Table**, relative to acetone (2.225 ppm for ^{1}H and 31.07 ppm for ^{13}C). H6, C6 of hexoses are in blue: those belonging to 6-linked hexoses resonate at about 67 ppm, those that are not linked at about 62 ppm.

Table B. ^{1}H and ^{13}C chemical shift assignments of Tramesan, referred to acetone (2.225 ppm for ^{1}H and 31.07 ppm for ^{13}C).

Residues	Nucleus	Chemical shifts (ppm)					
		1	**2**	**3**	**4**	**5**	**6**
A	^{1}H	5.35	4.12				
→2)-Man-(1→	^{13}C	101.46	79.03				
B	^{1}H	5.33	4.10				
→2)-Man-(1→	^{13}C	101.44	79.13				
C	^{1}H	5.30	4.11				
→2)-Man-(1→	^{13}C	101.43	79.03				
D	^{1}H	5.28	4.11				
→2)-Man-(1→	^{13}C	101.43	79.03				
E	^{1}H	5.27	4.08				
→2)-Man-(1→	^{13}C	101.43	79.24				
F	^{1}H	5.13	4.08				
Man-(1→	^{13}C	103.06	70.78				
G	^{1}H	5.12	3.93	3.97	3.84	4.17	1.24
→3)-α-Fuc-(1→	^{13}C	102.18	68.53	78.26	71.29	67.97	16.45
H	^{1}H	5.09	3.81				
	^{13}C	102.26	69.30				
I	^{1}H	5.07	4.07				
	^{13}C	102.97	69.25				
J	^{1}H	5.05	3.84				
	^{13}C	98.80	78.62				
K	^{1}H	5.01	3.85				
	^{13}C	98.91	78.52				

Table C. ^{1}H and ^{13}C chemical shift assignments of the disaccharides obtained from partial hydrolysis of Tramesan. Signals are referred to acetone (2.225 ppm for ^{1}H and 31.07 ppm for ^{13}C).

Residues	Nucleus	Chemical shifts (ppm)					
		1	**2**	**3**	**4**	**5**	**6**
A	^{1}H	5.26	3.80	3.78	4.03	4.26	3.69-3.84
→6)-α-Gal	^{13}C	93.31	71.68		70.29	69.35	67.24
B	^{1}H	5.21	3.88	3.95	3.86	4.22	1.18
→3)-α-Fuc	^{13}C	93.11	70.85	79.91	70.24	67.19	16.22
C	^{1}H	5.09	4.08	3.90	3.66	3.77	
Man-(1→	^{13}C	103.21	70.94	70.70	67.74	74.12	
D	^{1}H	4.97	3.82	3.86	3.97		
α-Gal-(1→	^{13}C	99.49	69.21	70.24	71.82		
E	^{1}H	4.60	3.56	3.73	3.90	3.81	1.22
→3)-β-Fuc	^{13}C	97.12	71.88	81.57	71.55	71.68	16.22
F	^{1}H	4.59	3.50	3.65	3.98		3.69-3.84
→6)-β-Gal	^{13}C	97.19	72.77	73.56	69.91		67.24

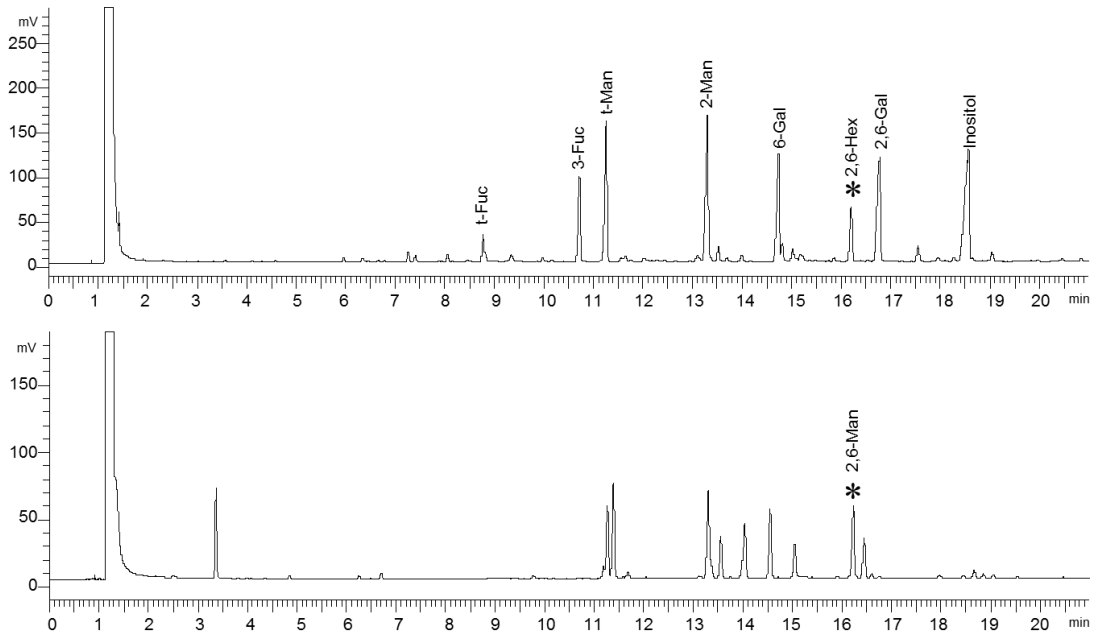

Figure H. Comparison of GLC elution profiles between PMAA derivatives of Tramesan and a sample containing 2,6-Man. The peaks of interest are marked with asterisks.