Supplementary material

Article

Water-soluble *Saccharina latissima* polysaccharides and relation of their structural characteristics with *in vitro* immunostimulatory and hypocholesterolemic activities

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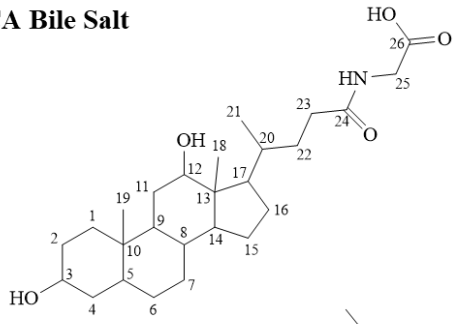
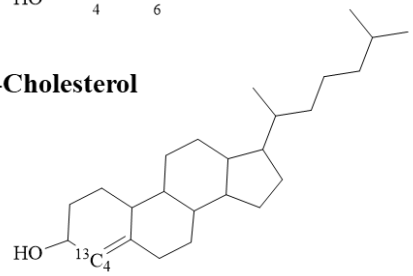
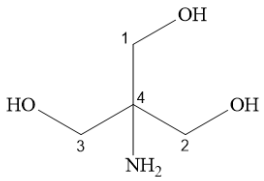
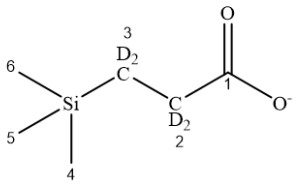
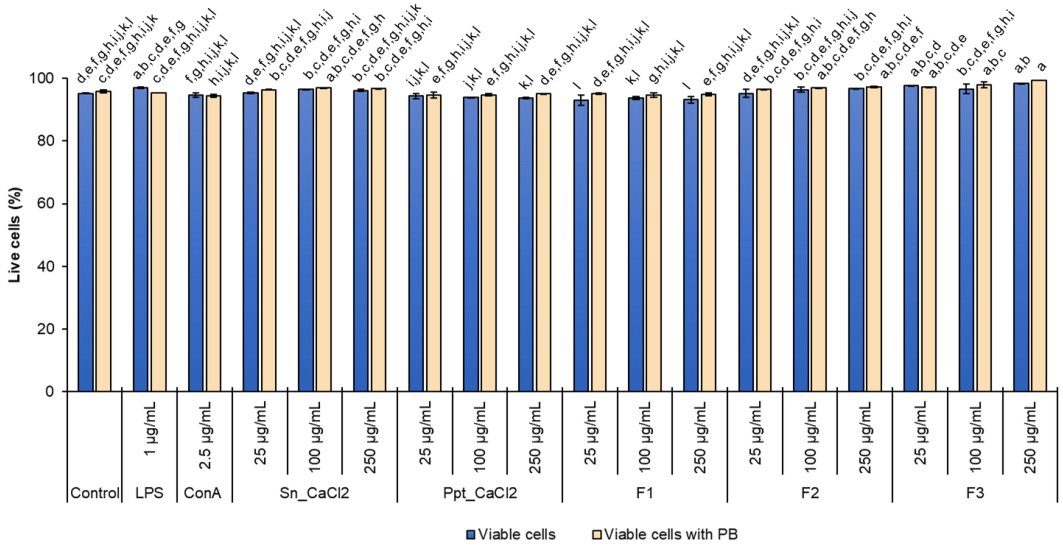
Supplementary Table S1. Content (%, w/w) of sulphur (S) and nitrogen (N) determined by elemental analysis, as well as sulphates (calculated as $-SO_3^-$) and proteins (calculated as N × 4.37).

Sample	Elemental analysis		Sulphates (calculated as $-SO_3^-$)	Proteins (N × 4.37)
	S	N		
Sn_$CaCl_2$	2.5 ± 0.03	2.6 ± 0.02	6.4 ± 0.1	11.2 ± 0.1
F1	-	0.6 ± 0.04	-	2.6 ± 0.2
F2	1.9 ± 0.3	4.7 ± 0.1	4.8 ± 0.4	20.7 ± 0.2
F3	5.7 ± 0.6	1.4 ± 0.01	14.3 ± 1.5	6.2 ± 0.02

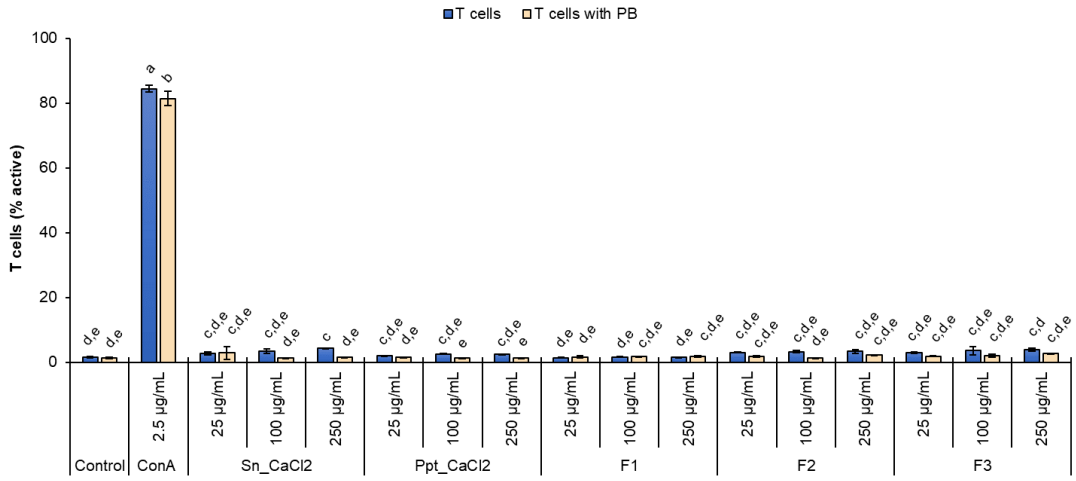
Data are presented as mean ± standard deviation.

Supplementary Table S2. ^{13}C NMR assignments of bile salt, cholesterol, Trizma buffer, and TSP standard resonances.

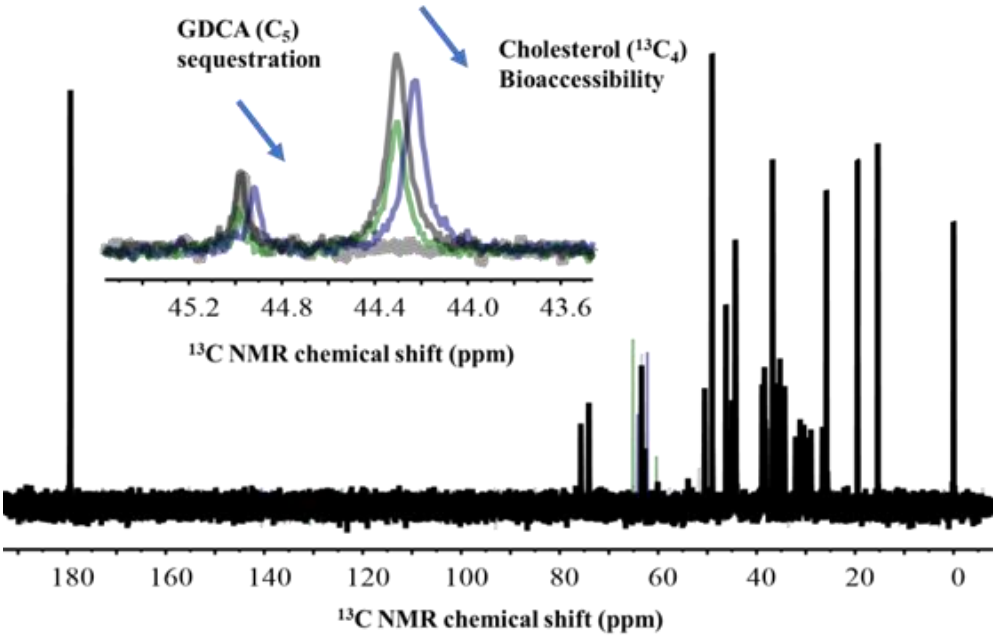
ppm	Assignments	Molecular Structure
179.5	GDCA, C_{24} (quaternary carbon)	
179.4	GDCA, C_{26} (quaternary carbon)	
75.7	GDCA, C_{12} (tertiary carbon)	**GDCA Bile Salt** 
74.1	GDCA, C_3 (tertiary carbon)	
63.3	Trizma, C_1, C_2, C_3 (methylene carbon)	
62.7	Trizma, C_4 (tertiary carbon)	
50.6	GDCA, C_{14} (tertiary carbon)	
49.1	GDCA, C_{17} (tertiary carbon)	
49.0	GDCA, C_{13} (quaternary carbon)	
46.2	GDCA, C_{25} (methylene carbon)	
45.0	GDCA, C_5 (tertiary carbon)	
44.3	Cholesterol, $^{13}C_4$ (tertiary carbon)	**$^{13}C_4$-Cholesterol** 
38.9	GDCA, C_8 (tertiary carbon)	
38.4	GDCA, C_{20} (tertiary carbon)	
38.2	GDCA, C_1 (methylene carbon)	
38.1	GDCA, C_4 (methylene carbon)	
36.8	GDCA, C_{10} (quaternary carbon)	
36.2	GDCA, C_9 (tertiary carbon)	**Trizma Buffer** 
35.3	GDCA, C_{23} (methylene carbon)	
34.3	GDCA, C_{22} (methylene carbon)	
32.1	GDCA, C_2 (methylene carbon)	
31.2	GDCA, C_{11} (methylene carbon)	
30.4	GDCA, C_{16} (methylene carbon)	**TSP standard** 
30.0	GDCA, C_6 (methylene carbon)	
29.0	GDCA, C_7 (methylene carbon)	
26.6	GDCA, C_{15} (methylene carbon)	
25.8	GDCA, C_{19} (methyl carbon)	
19.5	GDCA, C_{21} (methyl carbon)	
15.4	GDCA, C_{18} (methyl carbon)	
0.0	TSP, C_4, C_5, C_6 (methyl carbons)	



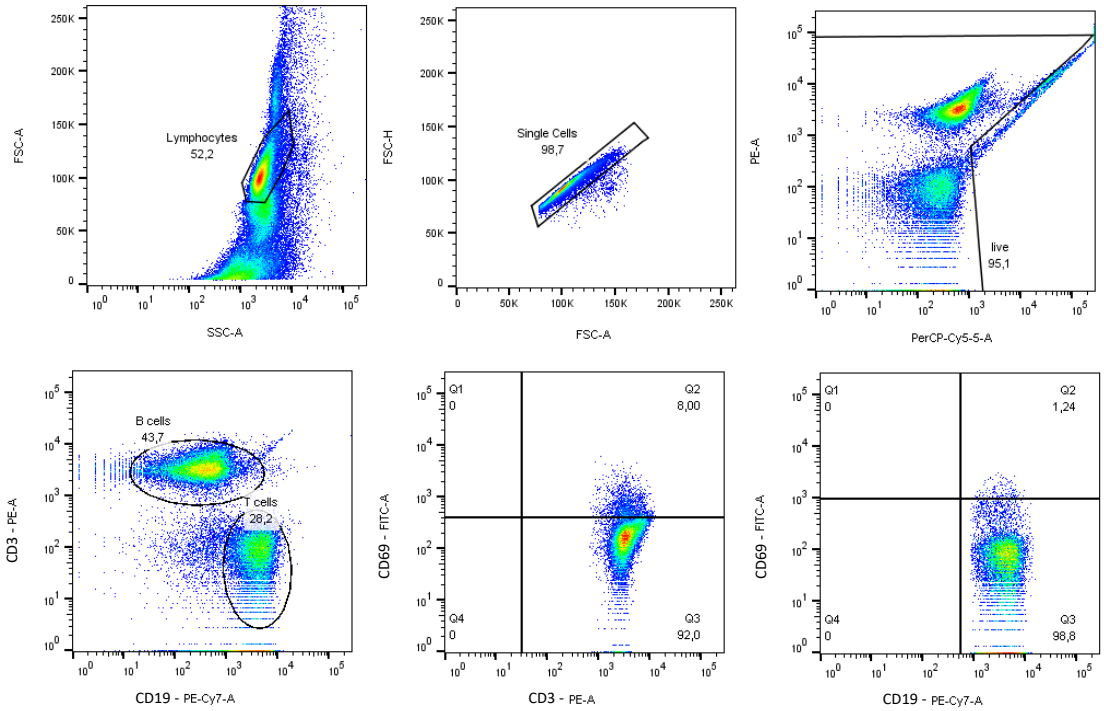
Supplementary Figure S1. Viable cells (%) cultured for 6 h with polysaccharide-enriched fractions obtained from *S. latissima* (Sn_$CaCl_2$, Ppt_$CaCl_2$, F1, F2, and F3) at the concentrations of 25, 100, and 250 µg/mL, in the absence and presence of polymyxin B (PB). Culture medium alone (RPMI) was used as negative control. Lipopolysaccharide (LPS) and concanavalin A (ConA) were used as positive controls. Mean (± SD) values are represented. Different letters above the bars indicate statistically significant differences between compared groups (p<0.05).



Supplementary Figure S2. Percentage of T cells activated by incubation with polysaccharide-enriched fractions obtained from *S. latissima* (Sn_$CaCl_2$, Ppt_$CaCl_2$, F1, F2, and F3) at the concentrations of 25, 100, and 250 µg/mL, in the absence and presence of polymyxin B (PB). Culture medium alone (RPMI) was used as negative control. Concanavalin A (ConA) was used as positive control. Mean (± SD) values are represented. Different letters above the bars indicate statistically significant differences between compared groups (p<0.05).



Supplementary Figure S3. Representative ^{13}C NMR spectrum of GDCA bile salt solution 50 mM (grey), GDCA bile salt solution 50 mM with labelled $^{13}C_4$ Cholesterol 3.5 mM (black) in the presence of cationic resin colestipol 5 mg/mL (green) or polysaccharide fucoidan (F2) 5 mg/mL (blue).



Supplementary Figure S4. Gating strategy for flow cytometry analysis. Representative dot plots are presented showing the gating strategy for lymphocytes (FCS vs SSC), non-aggregated cells (FCS-H vs FCS-A), live cells (propidium iodide), B and T cells (CD3 vs CD19), and activated cells (CD69 vs CD3 and CD69 vs CD19).