SUPPLEMENTARY MATERIAL

Evaluation of the anticoagulant potential of polysaccharide-rich fractions extracted from macroalgae

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Abstract: The aim of this study was to evaluate the potential anticoagulant activity of sulfated polysaccharide-containing extracts of six french edible marine macroalgae. Aqueous extracts of brown (*Himanthalia elongata*, *Laminaria digitata*, *Ascophyllum nodosum*, *Fucus vesiculosus*), green (*Ulva lactuca*), and red (*Chondrus crispus*) macroalgae, were prepared and their biochemical properties were determined, including major biomolecules, sulfate and ash contents. The anticoagulant activity of each extract was investigated using different scales, from the specific antithrombin-dependent pathway (anti-Xa and anti-IIa) to the intrinsic and/or common (Activated Partial Thromboplastin Time, APTT), extrinsic (Prothrombin Time, PT) or common (Thrombin Time, TT) anticoagulant pathways, and compared with those of commercial anticoagulants, heparin and Lovenox®. *Laminaria digitata*, *Fucus vesiculosus* and *Chondrus crispus* extracts showed a significant APTT anticoagulant capacity, only 5-fold lower than that of Lovenox®, which is a pure low molecular weight heparin used as an anticoagulant agent to prevent pulmonary embolism in patients undergoing surgery.

Keywords: Marine macroalgae, Phaeophyceae, Chlorophyceae, Rhodophyceae, aqueous extracts, anticoagulant activity

Table S1. Biochemical properties (wt%) and monosaccharide composition (molar ratio, %) of the seaweed extracts.

Seaweed extract	Neutral sugar content (%)	Uronic acid content (%)	Sulfates on the sugar backbone (%)	Protein content (%)	Polyphenol content (%)	Ash (%)	Monosaccharide composition (molar ratio, %)						
							Gal and/or Glc [(a)]	Xyl	Rha	Fuc	GlcA and/or IdoA [(a)]	GlcN	GlcNAc
Himanthalia elongata	16	19.4	12.8	13.7	4.8	14.1	14.6	4.3	13.4	50.3	13.4	1.9	2.1
Laminaria digitata	21	14.1	3.5	8	11.9	7.3	25.3	-	-	39.6	26.8	3.8	4.6
Ascophyllum nodosum	19.1	9.2	6.1	57	7.5	8.3	13.1	20.8	11.6	39.0	11.6	1.9	1.9
Fucus vesiculosus	16.4	7.9	7.9	36.9	5.1	9.6	16.4	7.4	-	70.0	-	2.6	3.6
Ulva lactuca	25.5	8.9	12.9	2.9	3.4	21.3	2.6	0.1	48.9	-	47.1	0.7	0.8
Chondrus crispus	19.2	2.3	9.4	14.4	1.4	13.5	96.4	-	-	-	-	1.5	2.1

[(a)] *These epimers could not be distinguished by UHPLC-HRMS analysis*

Table S2. TT analysis of the seaweed extracts, compared to Lovenox® and heparin.

	Extract concentration (µg.mL^{-1})	0.125	1.25	12.5	125
TT (s) [(a)]	*Himanthalia elongata*	17.3 ± 0.3	17.1 ± 0.4	20.0 ± 0.1	56.2 ± 3.6
	Laminaria digitata	16.9 ± 0.2	17.6 ± 0.5	28.3 ± 2.5	> 60
	Ascophyllum nodosum	17.3 ± 0.2	16.8 ± 0.6	24.0 ± 0.4	> 60
	Fucus vesiculosus	17.1 ± 0.6	17.4 ± 0.5	24.5 ± 0.6	> 60
	Ulva lactuca	16.9 ± 0.2	17.6 ± 0.5	28.3 ± 2.5	> 60
	Chondrus crispus	17.3 ± 0.6	17.5 ± 0.3	22.8 ± 0.4	> 60
	Lovenox®	17.3 ± 0.2	35.6 ± 1.3	> 60	-
	Heparin	18.5 ± 0.4	> 60	-	-

[(a)] *The clotting time of the negative control (0.9% NaCl) was 16.1 ± 0.6 s and the maximum recorded clotting time was 60 s.*

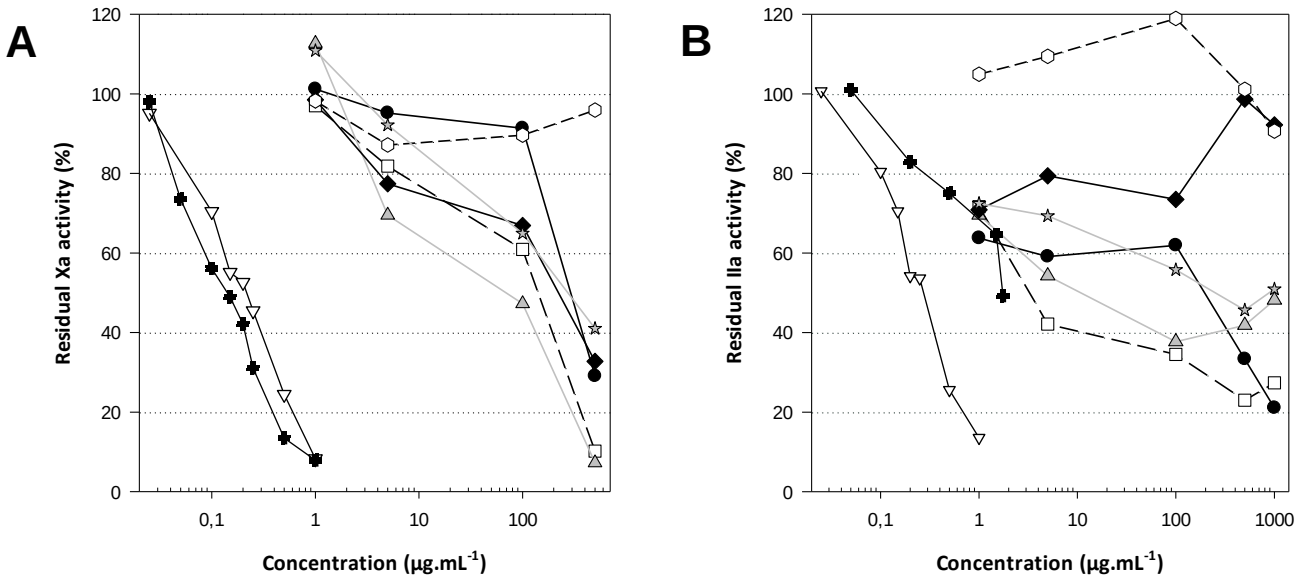

*Figure S1. Dose-response curves of anti-Xa (**A**) and anti-IIa (**B**) antithrombin-mediated activities of the selected seaweed extracts (Himanthalia elongata ●, Laminaria digitata □, Ascophyllum nodosum △, Fucus vesiculosus ◆, Ulva lactuca ⬡, Chondrus crispus ☆), compared to Lovenox® (✚) and heparin (▽).*

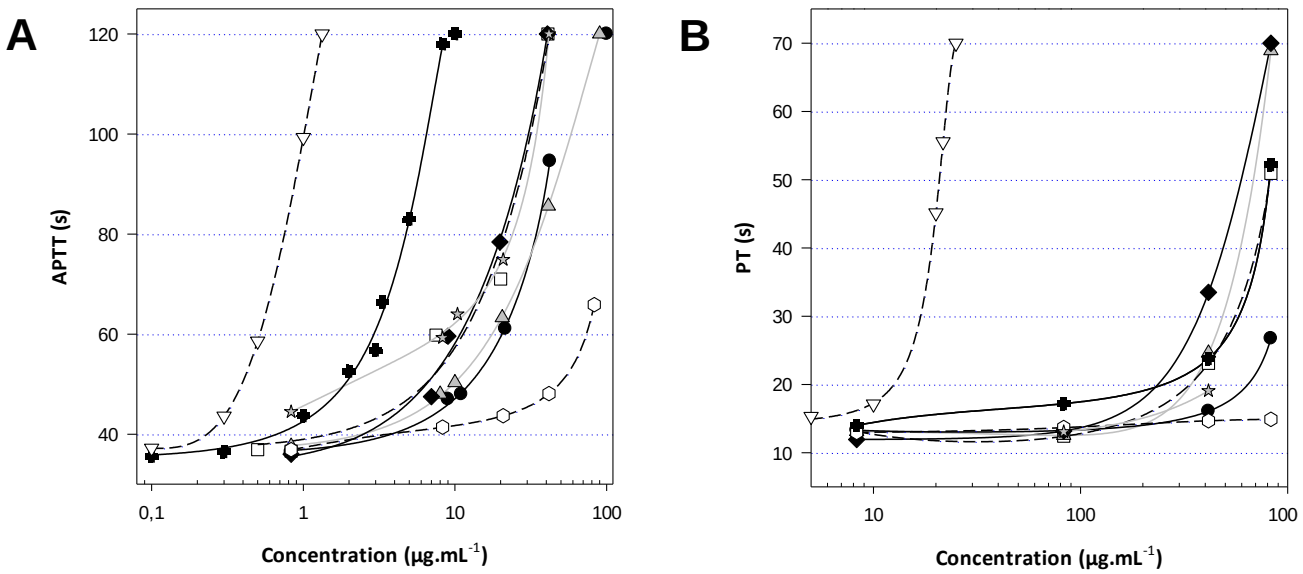

*Figure S2. Dose-response curves of APTT (**A**) and PT (**B**) analyses of the selected seaweed extracts (Himanthalia elongata ●, Laminaria digitata □, Ascophyllum nodosum △, Fucus vesiculosus ◆, Ulva lactuca ⬡, Chondrus crispus ☆), compared to Lovenox® (✤) and heparin (▽). The clotting times of the negative control (0.9% NaCl) were 38.2 s (APTT) and 13.1 s (PT). The maximum recorded clotting times were 120 s (APTT) and 70 s (PT).*