NEUROPROTECTIVE AND ANTIOXIDATIVE PROPERTIES OF *Sargassum hornschuchii*, BROWN MACROALGA FROM THE ADRIATIC SEA

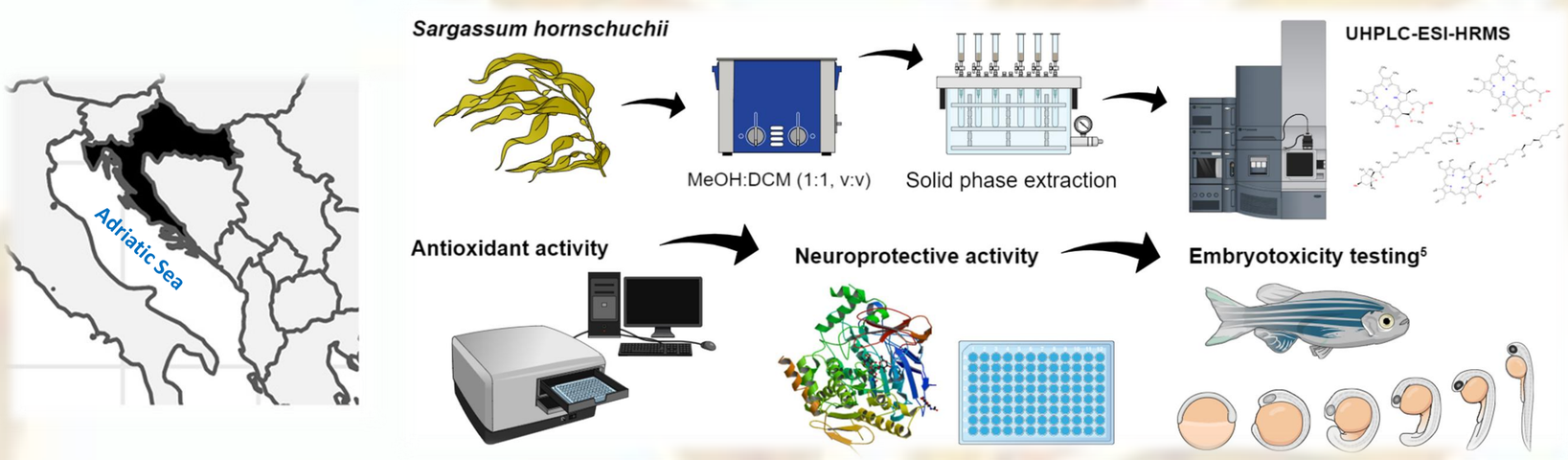
Sanja Babić Brčić[a,b], Tina Paradžik[a,b], Sanja Radman[c], Lara Čižmek[a,b], Ana-Marija Cikoš[d], Stela Jokić[d], Igor Jerković[c], Rozelindra Čož-Rakovac[a,b]

[a]Ruđer Bošković Institute, Zagreb, Croatia; [b]Center of Excellence for Marine Bioprospecting (BioProCro), Zagreb Croatia; [c]Faculty of Chemistry and Technology, University of Split, Croatia; [d]Faculty of Food Technology, Josip Juraj Strossmayer University of Osijek, Croatia; Correspondence to: babic@irb.hr

INTRODUCTION:

Marine macroalgae are rich sources of structurally diverse bioactive molecules with beneficial properties that hold potential for cosmeceutical, pharmaceutical and biomedical applications[1]. *S. hornschuchii* has been reported to harbor various bioactive substances with antioxidant, anticoagulation, and antibacterial properties[2,3].
However, these activities have been shown to vary based on sampling locations, seasons, environmental conditions, and extraction protocols. The challenging environment of the Adriatic Sea, characterized by high salinity, temperature fluctuations, and intense UV radiation, has driven macroalgae to develop unique bioactive compounds whose bioactivity is yet to be fully explored[4].

METHODS:



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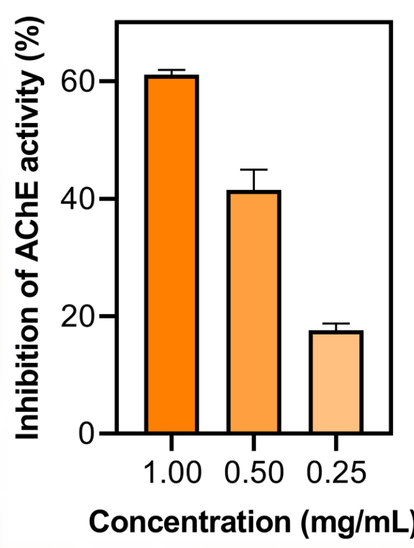
RESULTS:

CHEMICAL COMPOSITION

Table 1. Major non-volatile compounds in F3 and F4 fractions and their tentative identification by UHPLC-ESI(+)-HRMS.

No. (t_R)	Name	Mass	[M+H]+	MF	t_R (min)	Mass Difference (ppm)	F3 Area (counts)	F4 Area (counts)
	Fatty acid derivatives							
1	Loliolide	196.110	197.11722	$C_{11}H_{16}O_3$	5.8	2.8	4.26E+04	/
7	Tetradecanamide	227.225	228.23219	$C_{14}H_{29}NO$	12.5	8.4	1.60E+06	1.16E+06
8	Palmitoleamide	253.241	254.24784	$C_{16}H_{31}NO$	13.0	0.7	6.84E+06	5.07E+06
10	Palmitamide	255.256	256.26349	$C_{16}H_{33}NO$	13.7	10.7	1.05E+07	6.62E+06
2	Hexadecasphinganine	273.267	274.27406	$C_{16}H_{35}NO_2$	7.7	0.4	5.56E+06	2.49E+06
9	Linoleamide	279.256	280.26349	$C_{18}H_{33}NO$	13.4	2.2	9.45E+06	6.47E+06
12	Oleamide	281.272	282.27914	$C_{18}H_{35}NO$	14.1	0.4	9.43E+07	7.59E+07
16	Octadecanamide	283.288	284.29479	$C_{18}H_{37}NO$	14.8	6.1	2.75E+06	1.98E+06
14	Arachidonic acid	304.24	305.24751	$C_{20}H_{32}O_2$	14.8	6.0	8.27E+05	/
18	cis-11-Eicosenamide	309.303	310.31044	$C_{20}H_{39}NO$	15.1	4.6	1.43E+06	8.56E+05
11	Glyceryl palmitate	330.277	331.28429	$C_{19}H_{38}O_4$	14.0	5.1	2.03E+05	1.46E+05
22	Erucamide	337.334	338.34174	$C_{22}H_{43}NO$	16.0	0.3	8.32E+06	3.36E+06
17	Glycerol monostearate	358.308	359.31559	$C_{21}H_{42}O_4$	15.1	6.3	2.28E+06	1.86E+06
27	(2S)-1-Hydroxy-3-(tetradecanoyloxy)-2-propanyl (9Z)-9-octadecenoate	566.491	567.4983	$C_{35}H_{66}O_5$	17.9	8.2	2.57E+06	1.06E+06
26	Dipalmitin	568.507	569.51395	$C_{35}H_{68}O_5$	17.9	8.0	1.99E+05	8.94E+04
23	3-{[6-O-(α-D-Galactopyranosyl)-β-D-galactopyranosyl]oxy}-2-[(9Z)-9-hexadecenoyloxy]propyl (9Z,12Z,15Z)-9,12,15-octadecatrienoate	912.581	913.5883	$C_{49}H_{84}O_{15}$	17.0	5.4	1.11E+06	1.28E+05
	Terpenoids and steroids - derivatives							
4	Chola-5,22-dien-3-ol	342.292	343.29954	$C_{24}H_{38}O$	9.2	4.1	3.73E+05	2.13E+05
3	(3β,20E)-24-Norchola-5,20(22)-diene-3,23-diol	344.272	345.27881	$C_{23}H_{36}O_2$	9.1	6.4	5.65E+04	3.23E+04
5	Brassicasterol	398.355	399.36214	$C_{28}H_{46}O$	11.4	5.9	4.79E+04	1.65E+05
25	(3β)-3-Hydroxystigmast-5-en-7-one	428.365	429.37271	$C_{29}H_{48}O_2$	17.4	6.8	1.36E+06	8.34E+05
28	(3β,6α)-14-Methylergosta-8,24(28)-diene-3,6-diol	428.365	429.37271	$C_{29}H_{48}O_2$	18.3	8.0	1.65E+05	6.88E+04
23	24-Hydroperoxy-24-vinyl-cholesterol	444.36	445.36762	$C_{29}H_{48}O_3$	17.0	6.0	2.44E+05	2.95E+05
6	1'H-5Alpha-Cholest-2-eno[3,2-b]indole	459.387	460.39378	$C_{33}H_{49}N$	12.5	7.0	5.66E+03	4.28E+03
	Pigments and derivatives							
20	(2E)-3-[21-(Methoxycarbonyl)-4,8,13,18-tetramethyl-20-oxo-9,14-divinyl-3,4-didehydro-3-24,25-dihydrophorbinyl]acrylic acid	586.222	587.2289	$C_{35}H_{30}N_4O_5$	15.5	6.9	6.81E+05	1.84E+05
21	Chlorophyll c	588.237	589.24455	$C_{35}H_{32}N_4O_5$	15.5	5.6	4.78E+05	1.75E+05
19	Pheophorbide a	592.269	593.27585	$C_{35}H_{36}N_4O_5$	15.4	8.6	8.57E+05	1.08E+05
13	Pheophorbide b	606.248	607.25511	$C_{35}H_{34}N_4O_6$	14.7	0.9	2.26E+04	/
15	Fucoxanthin	658.423	659.43062	$C_{42}H_{58}O_6$	14.8	6.0	8.38E+04	/
31	Pheophytin a	870.566	871.5732	$C_{55}H_{74}N_4O_5$	20.1	8.3	7.34E+05	1.59E+06
29	Hydroxypheophytin a	886.561	887.56811	$C_{55}H_{74}N_4O_6$	19.9	7.7	9.65E+05	4.26E+05
30	13-Hydroxy-pheophytin a	902.556	903.56303	$C_{55}H_{74}N_4O_7$	19.9	7.0	2.41E+05	8.51E+05

NEUROPROTECTIVE ACTIVITY



The tested F3 fraction was found to effectively inhibit AChE. At 1.00, 0.50 and 0.25 mg/mL, the F3 fraction inhibited AChE activity by 61.11 ± 0.79, 41.48 ± 3.48 and 17.60 ± 1.18 %, respectively.

The F4 fraction was not tested, as DMSO (the solvent in which the F4 fraction was re-suspended) itself exhibits anti-AChE activity.

Figure 1. Acetylcholinesterase (AChE) inhibitory activity of *S. hornschuchii* F3 fraction. Data are expressed as mean SD (n = 3).

ANTIOXIDANT ACTIVITY

By employing the DPPH assay, the F3 fraction at 1 mg/mL exhibited moderate antioxidant activity, with an inhibition of 27.93 mg/g AAE. Similar antioxidant activity was obtained for 1 mg/mL of F3 fraction by implementing an ABTS assay (16.49 mmol/g TE).
F4 fraction showed no antioxidant activity.

EMBRYOTOXIC POTENTIAL

Fractions were dissolved in suitable solvents: MeOH for F3 and DMSO for F4 fraction. During the toxicity testing, the concentration of the solvent did not exceed 1%, with the maximum tested concentration being 50 µg/mL. No averse effects on zebrafish development and survival were recorded during the test.

DISCUSSION

- Results obtained on *S. hornschuchii* fractions suggest significant potential for preventing neurodegenerative diseases and stress-related conditions by reducing oxidative stress and inhibiting acetylcholinesterase
- The observed bioactivity of F3 fractions may be attributed to pigments and derivatives such as fucoxanthin, pheophorbide a,b, and pheophytin a
- Given the high genetic similarity between zebrafish and humans, our findings suggest that zebrafish are a valuable model for assessing biomolecule safety and predicting their mechanisms of action in humans

The results highlight the bioactive potential of Adriatic brown macroalgae. S. hornschuchii provides a natural source of bioactive compounds with potential applications in the food industry

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