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Optimization of bioactive compounds with antioxidant activity of *Himanthalia elongata* by microwave assisted extraction using Response surface methodology.

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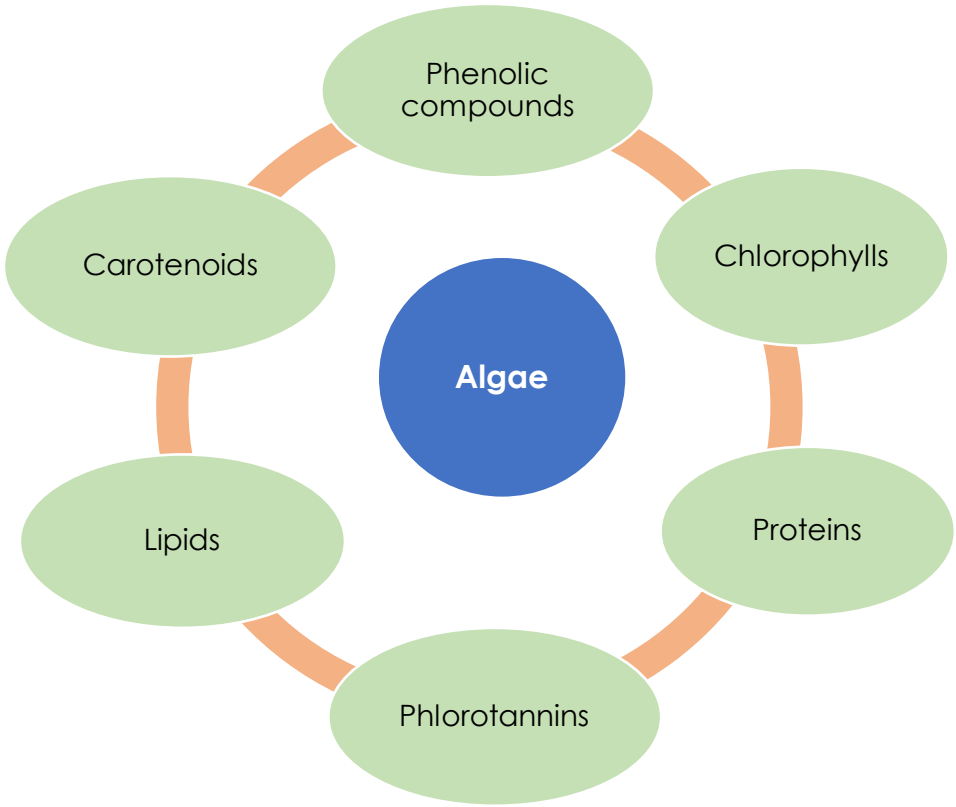
INTRODUCTION

Himanthalia elongata



Bioactivities

- Antioxidant
- Antimicrobial
- Antifungal
- Anti-inflammatory
- Antiproliferative cytotoxicity
- UV protection

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INTRODUCTION

- Traditionally, algae have been used as food and for medicinal purposes
- *Himanthalia elongata* is a brown algae of the order Fucales, found mainly in the N-W Atlantic Ocean and the North Sea.
- It is estimated that the value of the algae industry by 2025 will be 25,000 M $.

Industrial relevance

OBJECTIVES

Obtain extracts rich in compounds with potential functional properties.

Use "green" technologies to produce macroalgae extracts, contributing to sustainable exploitation.

Optimize the parameters (t, P and Et) that affect the extraction of bioactive compounds from *Himanthalia elongata* using the Response Surface Methodology (RSM) and simultaneously maximize the radical scavenging activity and the total phenol content.

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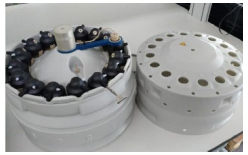
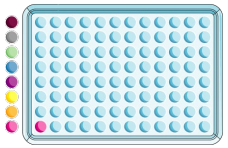
METHODOLOGY

Lyophilized

Storage at -20 ºC





Lyophilized Algae

T, P, % Et variables
Pw: 1400 W

Microwave extraction

Ice bath for 5 min

Cooling

9000 rpm for 15 min

Centrifugation

Filter paper

Filtration

-80 ºC for 24h

Storage

Extract rich in bioactive compounds

METHODOLOGY

- Extract rich in bioactive compounds
  - Yield (% DW)
  - Content of bioactive compounds
    - Total phenolic content
    - Total flavonoids content
  - Antioxidant activity
    - DPPH scavenging capacity
    - Antioxidant capacity of Trolox equivalents

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METHODOLOGY

Table 1. Experimental design for optimization

	Independet variables					
	X_1: t (min)		X_2: P(bar)		X_3: Et (% v/v)	
1	-1	(7,5)	-1	(5,6)	-1	(20,3)
2	-1	(7,5)	-1	(5,6)	1	(79,7)
3	-1	(7,5)	1	(16,4)	-1	(20,3)
4	-1	(7,5)	1	(16,4)	1	(79,7)
5	1	(20,5)	-1	(5,6)	-1	(20,3)
6	1	(20,5)	-1	(5,6)	1	(79,7)
7	1	(20,5)	1	(16,4)	-1	(20,3)
8	1	(20,5)	1	(16,4)	1	(79,7)
9	-1,68	(3)	0	(11)	0	(50)
10	1,68	(25)	0	(11)	0	(50)
11	0	(14)	-1,68	(2)	0	(50)
12	0	(14)	1,68	(20)	0	(50)
13	0	(14)	0	(11)	-1,68	(0)
14	0	(14)	0	(11)	1,68	(100)
15	-1,68	(3)	-1,68	(2)	-1,68	(0)
16	-1,68	(3)	-1,68	(2)	1,68	(100)
17	-1,68	(3)	1,68	(20)	-1,68	(0)
18	-1,68	(3)	1,68	(20)	1,68	(100)
19	1,68	(25)	-1,68	(2)	-1,68	(0)
20	1,68	(25)	-1,68	(2)	1,68	(100)
21	1,68	(25)	1,68	(20)	-1,68	(0)
22	1,68	(25)	1,68	(20)	1,68	(100)
23	0	(14)	0	(11)	0	(50)
24	0	(14)	0	(11)	0	(50)
25	0	(14)	0	(11)	0	(50)
26	0	(14)	0	(11)	0	(50)
27	0	(14)	0	(11)	0	(50)
28	0	(14)	0	(11)	0	(50)

CIRCUMSCRIBED CENTRAL COMPOSITE DESIGN (CCCD)

- 5 levels
- 3 coded variables

28 experimental points

Independent variables studied:

- Time (X_1): 3 – 25 min
- Pressure (X_2): 2 – 20 bar
- EtOH concentration (X_3): 0 – 100 % (aqueous-ethanol, v/v)

Solid - liquid ratio: 30 g/L

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5	1 (20,5)	-1 (5,6)	-1 (20,3)
6	1 (20,5)	-1 (5,6)	1 (79,7)
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8	1 (20,5)	1 (16,4)	1 (79,7)
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27	0 (14)	0 (11)	0 (50)
28	0 (14)	0 (11)	0 (50)

Response Surface Methodology (RSM)

$$Y = \overbrace{b_0}^{\text{Intercept}} + \underbrace{\sum_{i=1}^{n} b_i X_i}_{\text{Linear effect}} + \underbrace{\sum_{i=1}^{n-1}\sum_{\substack{j=2 \\ j>i}}^{n} b_{ij} X_i X_j}_{\text{Interactive effect}} + \underbrace{\sum_{i=1}^{n} b_{ii} X_i^2}_{\text{Quadratic effect}}$$

Response variables (dependent):

Y_1: % of extraction yield

Y_2: mg of total phenolics per g of dried seaweed

RESULTS

Table 2. Experimental results of 28 experiments for the optimization process.

	Independent variables						*Response variables*				
Run	***t* (min)**		***P* (bar)**		***Et* (%)**		**EY**	**TPC**	**TFC**	**DPPH**	**ABTS**
1	-1	(7.5)	-1	(5.6)	-1	(20.3)	452	24.58	2.60	5.64	12.24
2	-1	(7.5)	-1	(5.6)	1	(79.7)	355	28.26	6.14	6.59	80.08
3	-1	(7.5)	1	(16.4)	-1	(20.3)	529	41.30	4.28	19.97	21.38
4	-1	(7.5)	1	(16.4)	1	(79.7)	376	25.52	5.31	5.33	20.41
5	1	(20.5)	-1	(5.6)	-1	(20.3)	543	19.24	2.36	4.47	9.43
6	1	(20.5)	-1	(5.6)	1	(79.7)	384	23.90	5.11	6.29	22.01
7	1	(20.5)	1	(16.4)	-1	(20.3)	489	38.86	5.59	19.68	19.70
8	1	(20.5)	1	(16.4)	1	(79.7)	361	19.75	4.40	3.84	15.87
9	-1.68	(3)	0	(11)	0	(50)	459	25.56	2.92	4.70	22.12
10	1.68	(25)	0	(11)	0	(50)	370	37.70	4.02	7.86	35.70
11	0	(14)	-1.68	(2)	0	(50)	358	28.40	4.62	8.56	59.45
12	0	(14)	1.68	(20)	0	(50)	479	35.49	5.51	14.32	30.33
13	0	(14)	0	(11)	-1.68	(0)	491	25.50	11.31	11.28	15.12
14	0	(14)	0	(11)	1.68	(100)	109	11.89	4.19	5.66	22.58
15	-1.68	(3)	-1.68	(2)	-1.68	(0)	373	7.53	1.35	1.01	16.08
16	-1.68	(3)	-1.68	(2)	1.68	(100)	60	12.87	0.73	4.11	16.08
17	-1.68	(3)	1.68	(20)	-1.68	(0)	409	27.64	7.44	10.34	23.34
18	-1.68	(3)	1.68	(20)	1.68	(100)	99	10.14	4.61	3.57	7.22
19	1.68	(25)	-1.68	(2)	-1.68	(0)	459	8.10	1.92	1.05	74.57
20	1.68	(25)	-1.68	(2)	1.68	(100)	67	5.36	2.73	2.77	7.44
21	1.68	(25)	1.68	(20)	-1.68	(0)	443	29.75	9.20	13.43	37.66
22	1.68	(25)	1.68	(20)	1.68	(100)	133	3.89	7.78	3.95	8.18
23	0	(14)	0	(11)	0	(50)	377	35.62	2.62	9.58	61.86
24	0	(14)	0	(11)	0	(50)	377	32.35	4.26	9.11	60.83
25	0	(14)	0	(11)	0	(50)	474	47.73	9.64	25.29	95.13
26	0	(14)	0	(11)	0	(50)	425	21.61	3.29	5.60	24.28
27	0	(14)	0	(11)	0	(50)	439	21.89	3.70	4.50	22.10
28	0	(14)	0	(11)	0	(50)	435	21.97	3.44	4.97	23.59

RESULTS

Table 3. Effect of *H. elongata* extract by MAE under optimal conditions on antioxidant activity.

Best operating conditions	***%Et***	***P* (bar)**	***T* (min)**	
	0.00 ± 0.00	20.00 ± 0.50	16.01 ± 4.80	
EY mg/g of dw	**TPC** mg PGE/g dw	**TFC** mg QE/g dw	**Antioxidant Activity**	
			DPPH mg/mL	ABTS mg/mL
502.28 ± 25.11	37.43± 3.74	9.93 ±0.99	16.37±0.82	65.77 ± 1.97

CONCLUSION

Aqueous microwave-assisted extraction **(20 bar, 16 min)** appears to be the optimal processing method for the extraction of polyphenols with antioxidant activity from *Himanthalia elongata* without the need for organic solvents.

These are promising results because the polyphenol content was maximized using an environmentally friendly technique free of organic solvents.