Electronic Supporting Information

Efficient extraction and purification of mycosporines-like amino acids (MAAs) following a multiproduct biorefinery approach

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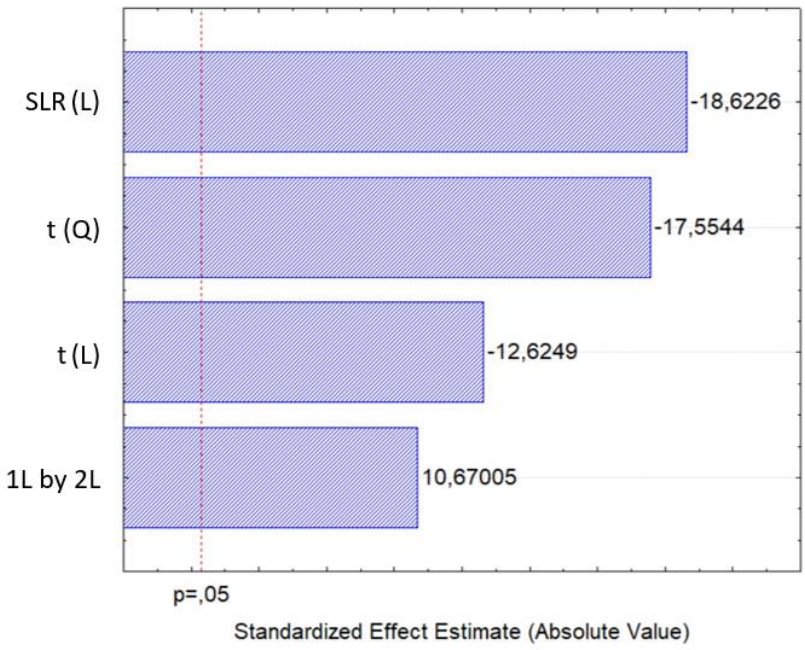

Figure S1. Pareto Chart of the CCRD (2^2) regarding MAAs yield of extraction ($mg_{MAAs}.g_{fresh\ biomass}^{-1}$) using PBS (1X, pH 7.4).

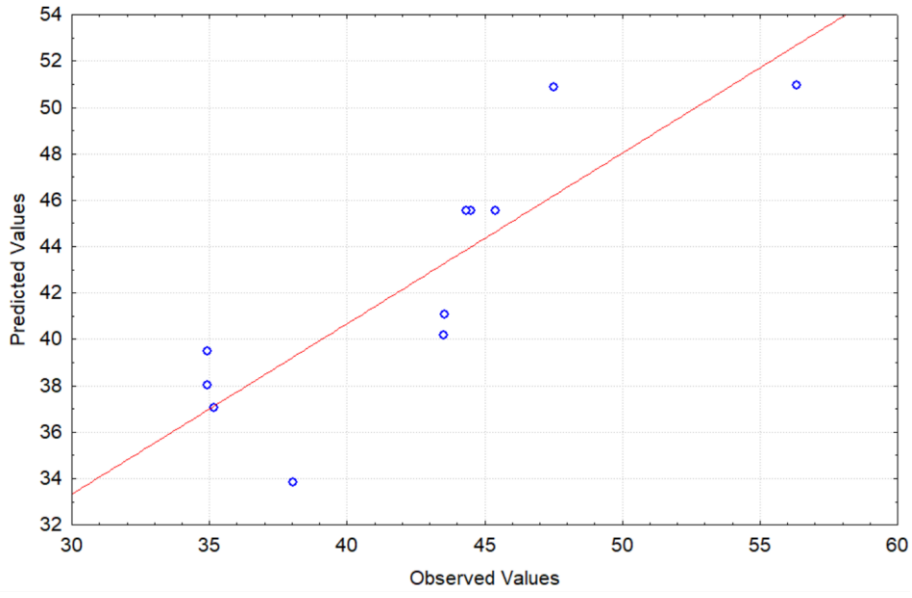

Figure S2. Predicted *vs.* experimental values of the CCRD (2^2) regarding MAAs yield of extraction ($mg_{MAAs}.g_{fresh\ biomass}^{-1}$) using PBS (1X, pH 7.4).

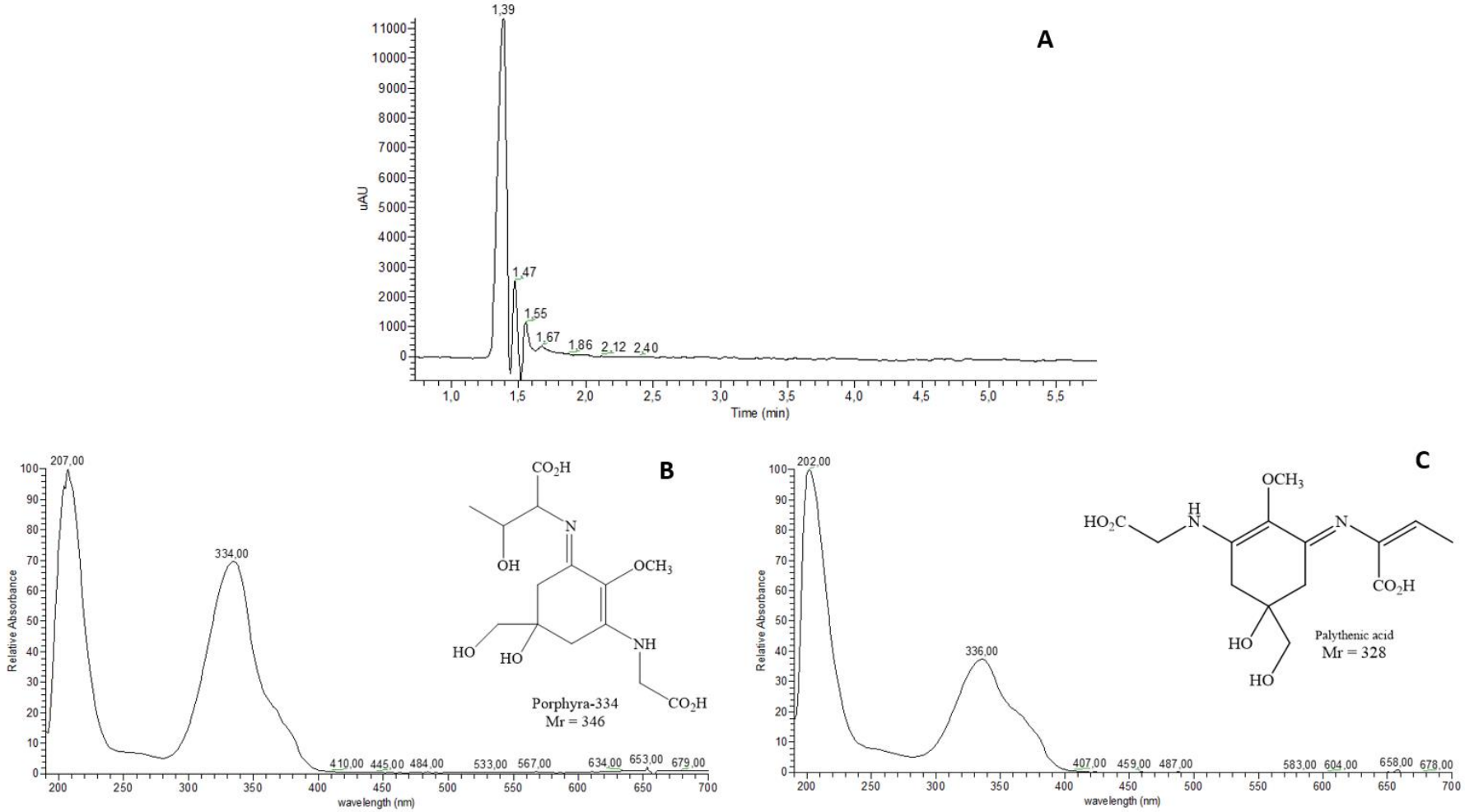

Figure S3. UHPLC-DAD-ESI/MS chromatogram of the resulting MAAs extract in PBS (1X, pH 7.4) (A), and respective UV-Vis spectra of the proposed compounds, namely porphyra-334 at a retention time of 1.39 min (B), and palythenic acid at a retention time of 1.47 min (C).

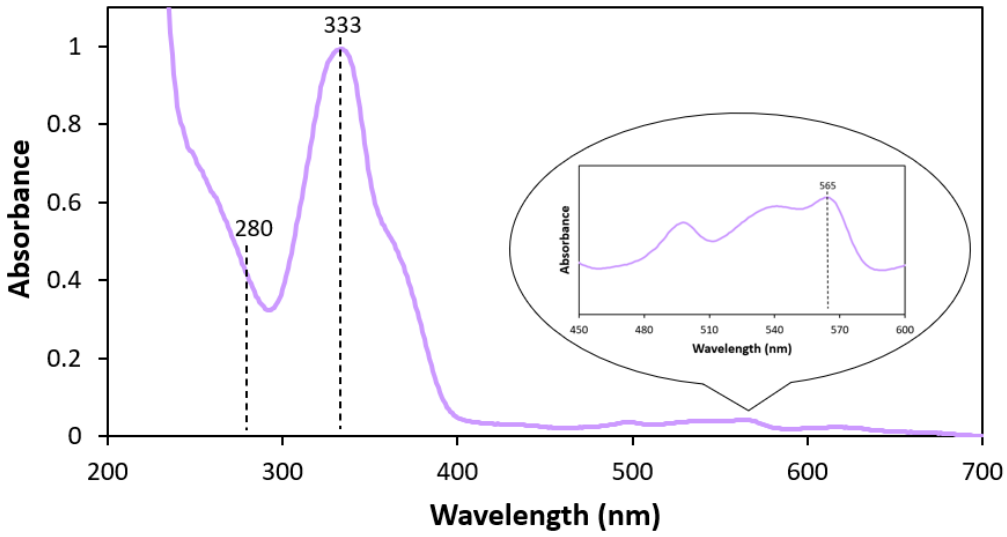

Figure S4. UV-Vis spectra profile of the recovered extract in PBS (1X, pH 7.4) diluted 10x using the optimized operating conditions previously obtained by CCRD (2^2). The dashed vertical lines represent the characteristic wavelengths of non-fluorescent proteins (280 nm), MAAs (333 nm), and R-phycoerythrin (565 nm).

Table S1. Real and encoded values used in the optimization process by CCRD (2^2) expressed by MAAs yield of extraction ($mg_{MAAs}.g_{fresh\ biomass}^{-1}$) using PBS (1X, pH 7.4).

Run	t (min)	SLR ($g_{fresh\ biomass}$. $mL_{solvent}^{-1}$)	Yield of extraction ($mg_{MAAs}.g_{fresh\ biomass}^{-1}$)
1	12.0 (-1)	0.180 (-1)	47.5
2	48.0 (1)	0.180 (-1)	34.9
3	12.0 (-1)	0.820 (1)	35.2
4	48.0 (1)	0.820 (1)	34.9
5	4.62 (-1.41)	0.500 (0)	43.6
6	55.4 (1.41)	0.500 (0)	38.0

7	30.0 (0)	0.0488 (-1.41)	56.3
8	30.0 (0)	0.951 (1.41)	43.5
9	30.0 (0)	0.500 (0)	44.5
10	30.0 (0)	0.500 (0)	44.3
11	30.0 (0)	0.500 (0)	45.4

Table S2. Effect of the estimates for MAAs yield of extraction ($mg_{MAAs}.g_{fresh\ biomass}^{-1}$) using PBS (1X, pH 7.4), optimized by the CCRD (2^2) with significant factors at 95% confidence level.

Factor	**Effect**	**Standard error**	**Calculated *t*** *	***p*-value**
Mean/Interaction	45.6	0.244	186	0.000
t (min) – (X1)	-5.21	0.413	-12.6	0.00622
t (min) – ($X1^2$)	-8.41	0.479	-17.6	0.00323
SLR ($g_{fresh\ biomass}.mL_{solvent}^{-1}$) – (X2)	-7.64	0.410	-18.6	0.00287
X1 by X2	6.17	0.578	10.7	0.00867

*Degrees of freedom.

Table S3. Predicted *vs.* experimental values (real) obtained by the fitted model and the respective relative deviation (%) from the independent variables fixed at the optimum conditions for MAAs yield of extraction ($mg_{MAAs}.g_{fresh\ biomass}^{-1}$) using PBS (1X, pH 7.4). V1, V2, and V3 represent the validation assays.

Assay	t (min)	SLR ($g_{fresh\ biomass}$. $mL_{solvent}^{-1}$)	Yield of extraction ($mg_{MAAs}.g_{fresh\ biomass}^{-1}$)		Relative deviation (%)
			Experimental values	Predicted values	
	X1	X2	Y	Predicted Y	
V1	12.0	0.0488	55.8	53.7	3.95
V2			59.6		11.0
V3			58.5		8.90
Mean of deviation					7.95

Table S4. Composition of the initial extract in PBS pH 7.4 (after solid-liquid extraction optimization) performed by UHPLC-DAD-ESI/MS.

Retention time (min)	UV-Vis (nm)	$[M-H]^-$ (*m/z*)	Compound	Compound abundance (%)
1.39	207, 334	345	Porphyra-334	80.63
1.47	202, 336	327	Palythenic acid	5.54