

## Electronic Supporting Information (ESI)

### **Unveiling the use of hydrophobic eutectic solutions as task-specific solvents to recover bacterioruberin from *Haloferax mediterranei***

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Number of pages: 7

Number of Tables: 3

Number of Figures: 8

**Table S1.** Real and coded values of the optimization process expressed by the yield of extraction of bacterioruberin by CCRD (2<sup>3</sup>) using Ment+LevA HES.

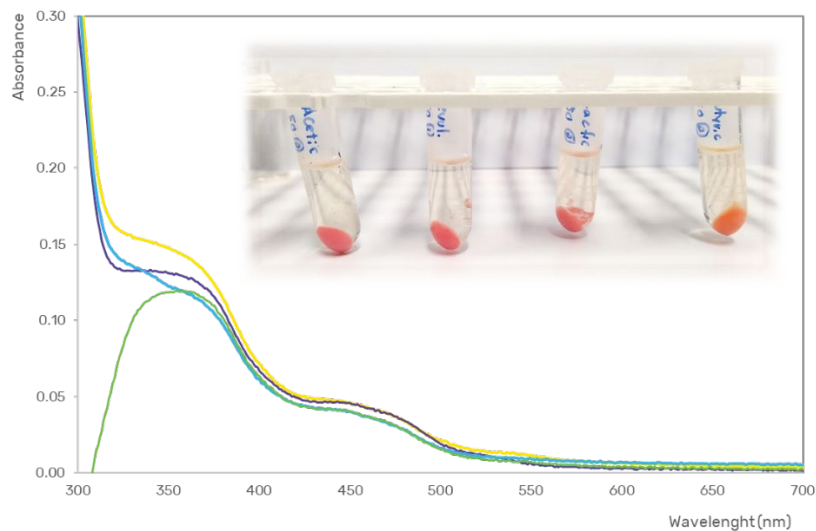
Run	$x_{\text{Ment}}$	SLR ( $\text{g}_{\text{wet biomass}} \cdot \text{mL}_{\text{solvent}}^{-1}$ )	Time (min)	Yield ( $\text{mg}_{\text{bacterioruberin}} \cdot \text{g}_{\text{wet biomass}}^{-1}$ )
	X1	X2	X3	Y1
1	-1 (0.54)	-1 (0.07)	-1 (30)	4.30
2	1 (0.66)	-1 (0.07)	-1 (30)	2.04
3	-1 (0.54)	1 (0.23)	-1 (30)	2.42
4	1 (0.66)	1 (0.23)	-1 (30)	1.62
5	-1 (0.54)	-1 (0.07)	1 (90)	4.47
6	1 (0.66)	-1 (0.07)	1 (90)	2.63
7	-1 (0.54)	1 (0.23)	1 (90)	3.35
8	1 (0.66)	1 (0.23)	1 (90)	1.99
9	-1.68 (0.50)	0 (0.15)	0 (60)	4.35
10	1.68 (0.70)	0 (0.15)	0 (60)	1.62
11	0 (0.60)	-1.68 (0.02)	0 (60)	3.28
12	0 (0.60)	1.68 (0.28)	0 (60)	1.63
13	0 (0.60)	0 (0.15)	-1.68 (9.6)	2.23
14	0 (0.60)	0 (0.15)	1.68 (110.4)	3.55
15	0 (0.60)	0 (0.15)	0 (60)	2.87
16	0 (0.60)	0 (0.15)	0 (60)	2.84
17	0 (0.60)	0 (0.15)	0 (60)	2.26
18	0 (0.60)	0 (0.15)	0 (60)	2.75
19	0 (0.60)	0 (0.15)	0 (60)	2.87
20	0 (0.60)	0 (0.15)	0 (60)	2.90

**Table S2.** Predicted results compared to the experimental values (real) obtained by the fitted model and the respective relative deviation (%) from the independent variables fixed at the optimum conditions of the surface points for the bacterioruberin extraction yield. V1, V2, and V3 represent the validation assays.

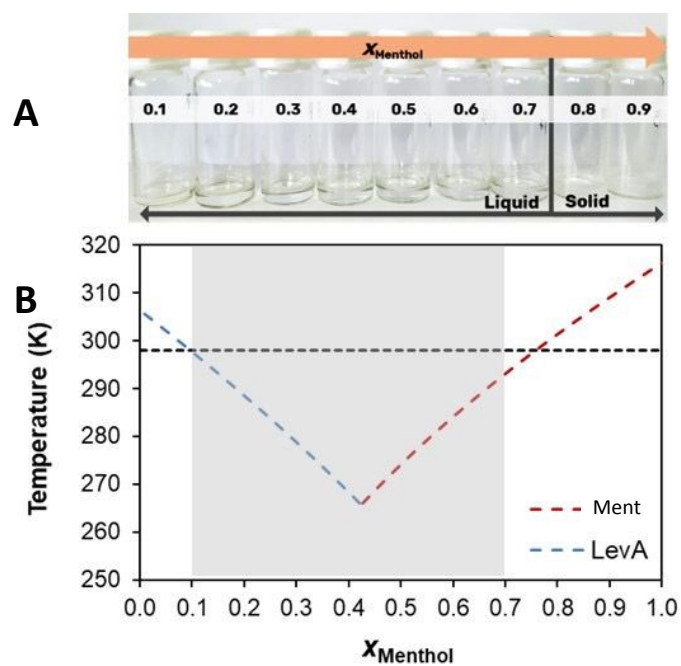
Assay	$x_{Ment}$	SLR ( $g_{wet\ biomass} \cdot mL_{solvent}^{-1}$ )	Time (min)	Yield ( $mg_{bacterioruberin} \cdot g_{wet\ biomass}^{-1}$ )		$\sigma$ (%)
				Experimental	Predicted	
V1				4.93		1.31
V2	0.54	0.07	110.4	4.51	4.86	-7.86
V3				4.80		-1.29
<b>Mean of deviation</b>						-2.61

**Table S3.** Predicted results compared to the experimental values (real) obtained by the fitted model and the respective relative deviation (%) from the independent variables fixed at the optimum conditions chosen for the continuation of the work. P1, P2, and P3 represent the chosen assays.

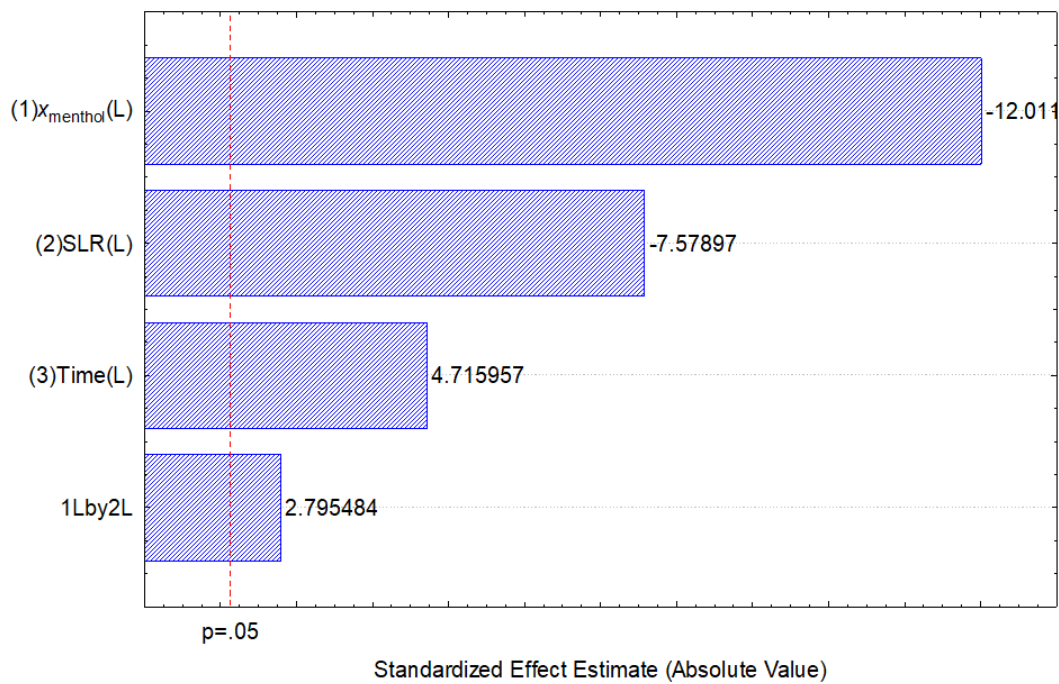
Assay	$x_{Ment}$	SLR ( $g_{wet\ biomass} \cdot mL_{solvent}^{-1}$ )	Time (min)	Yield ( $mg_{bacterioruberin} \cdot g_{wet\ biomass}^{-1}$ )		$\sigma$ (%)
				Experimental	Predicted	
P1				5.36		2.80
P2	0.50	0.10	110.4	4.92	5.21	-6.00
P3				5.21		-0.07
<b>Mean of deviation</b>						-1.09



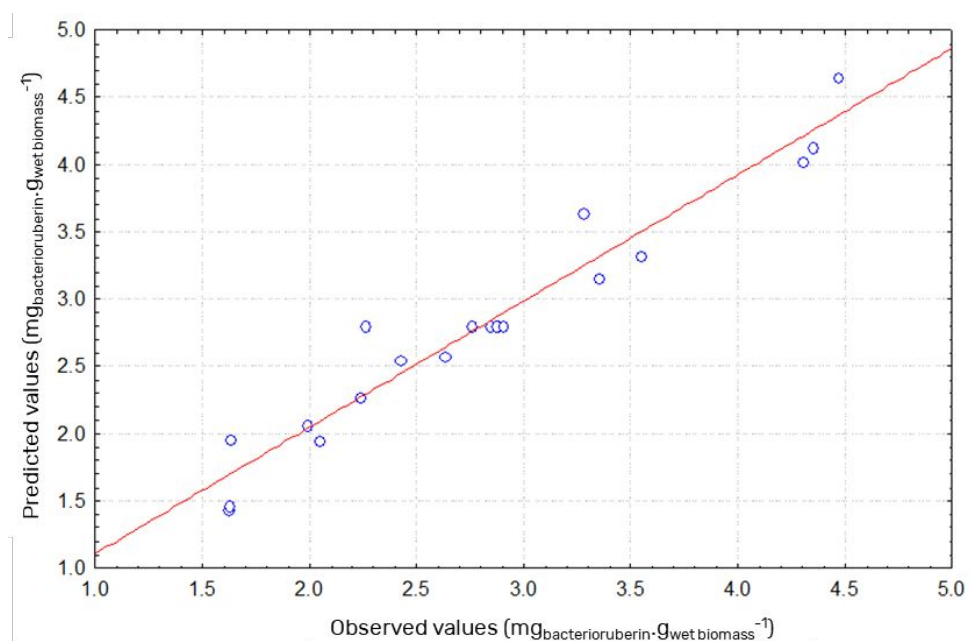
**Figure S1.** Spectra of the extracts from *H. mediterranei* using 50 vol.% carboxylic acid solutions (AcA, ButA, LA and LevA) in water.



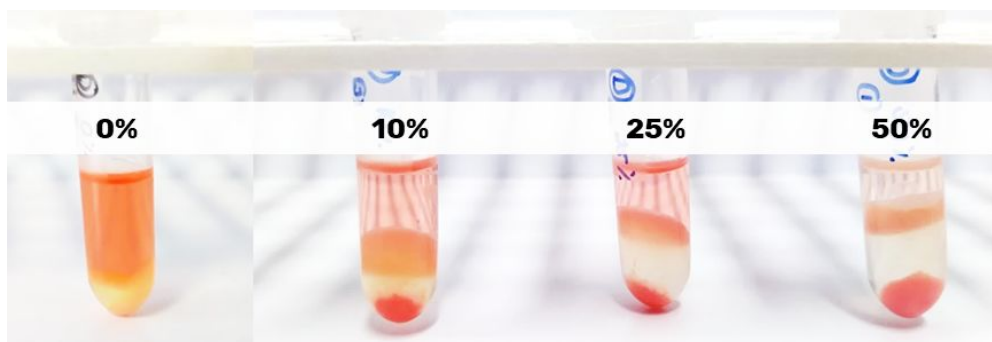
**Figure S2.** The liquidus range of Ment+LevA HES at room temperature, determined by preparing the binary mixture along the full composition range at  $x_{Ment}$  (A); and the ideal phase diagram of the Ment+LevA system.



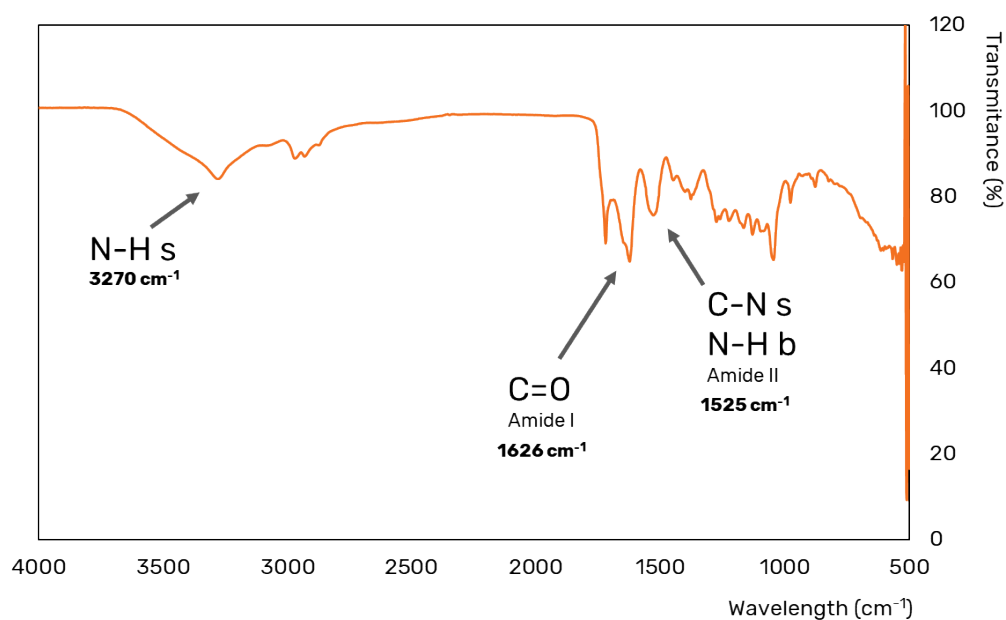
**Figure S3.** Pareto Chart of the CCRD ( $2^3$ ) regarding bacterioruberin yield of extraction ( $\text{mg}_{\text{bacterioruberin}} \cdot \text{g}_{\text{wet biomass}}^{-1}$ ) using Ment+LevA HES.



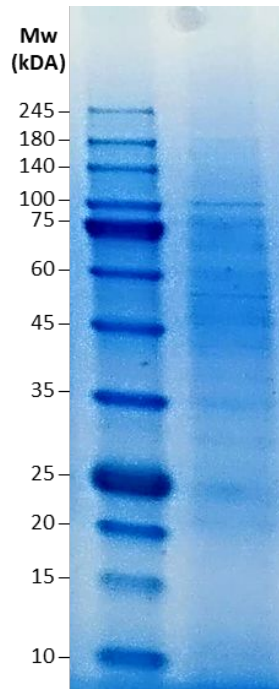
**Figure S4.** Predicted vs. experimental values of the CCRD ( $2^3$ ) regarding bacterioruberin yield of extraction ( $\text{mg}_{\text{bacterioruberin}} \cdot \text{g}_{\text{wet biomass}}^{-1}$ ) using Ment+LevA HES.



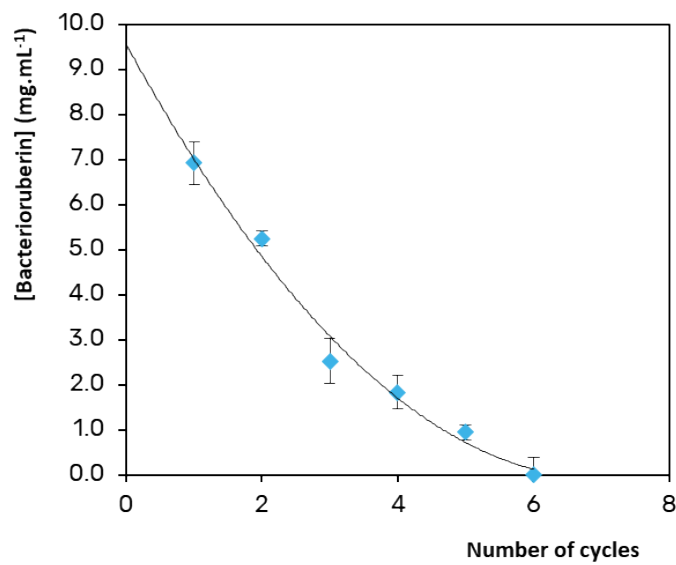
**Figure S5.** Photograph of the initial screening of the Ment+LevA HES and water systems for the extraction of bacterioruberin, where a white interfacial precipitate is detected.



**Figure S6.** FTIR spectra of the solid interface obtained from the system: Ment+LevA HES and water.



**Figure S7.** SDS-PAGE of the recovered proteins redissolved in PBS after protein precipitation.



**Figure S8.** Bacterioruberin yield of extraction obtained from consecutive extractions using Ment:LevA (◆). A higher yield of extraction is found on the first cycle when compared with the optimized value obtained previously due to slight differences in the biomass batch.