Electronic Supporting Information

Recovery of bacterioruberin and proteins using aqueous solutions of surface-active compounds

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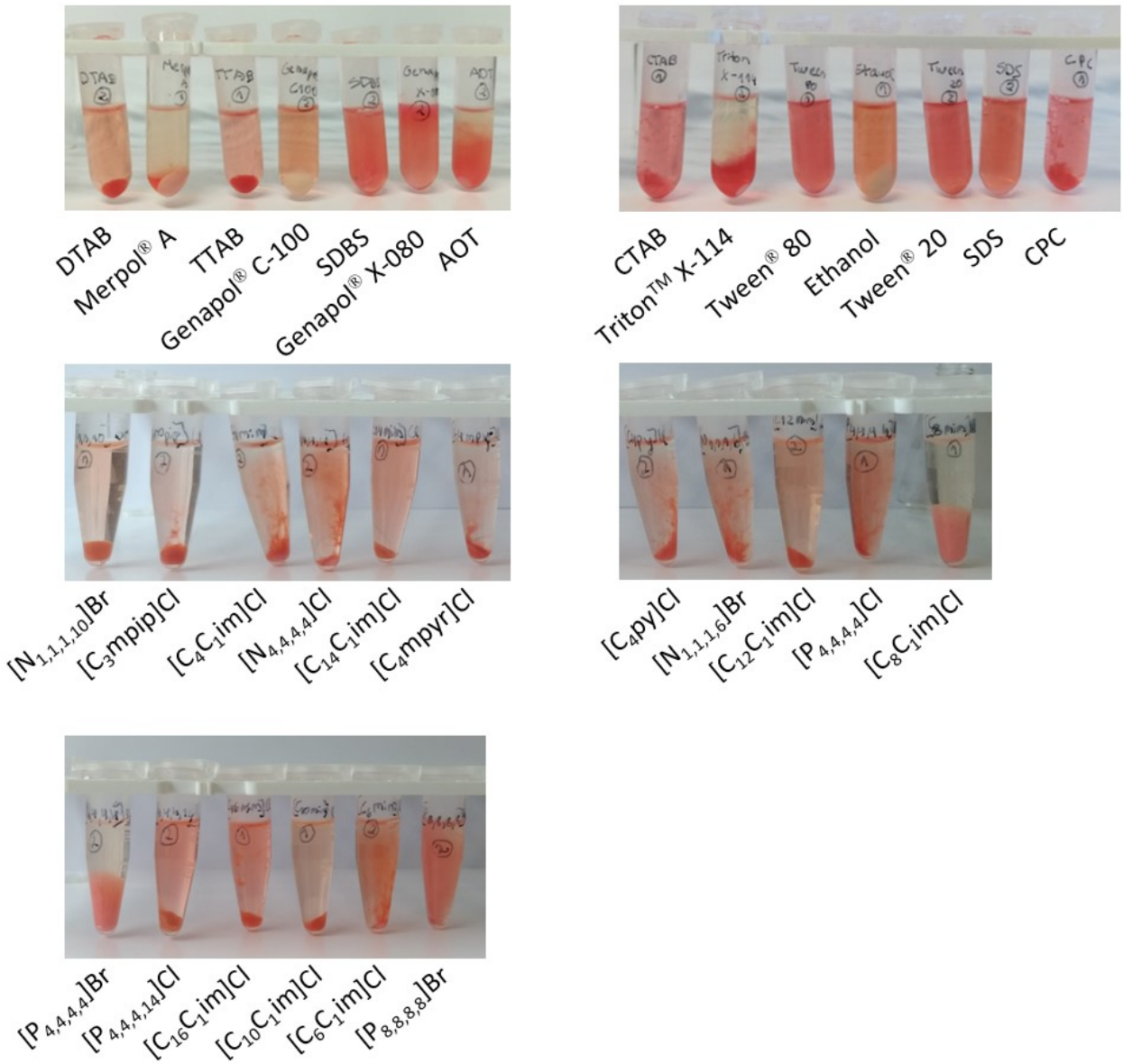


Fig. S1. Photographs of the extracts obtained in the screening of solvents at 100 mM.

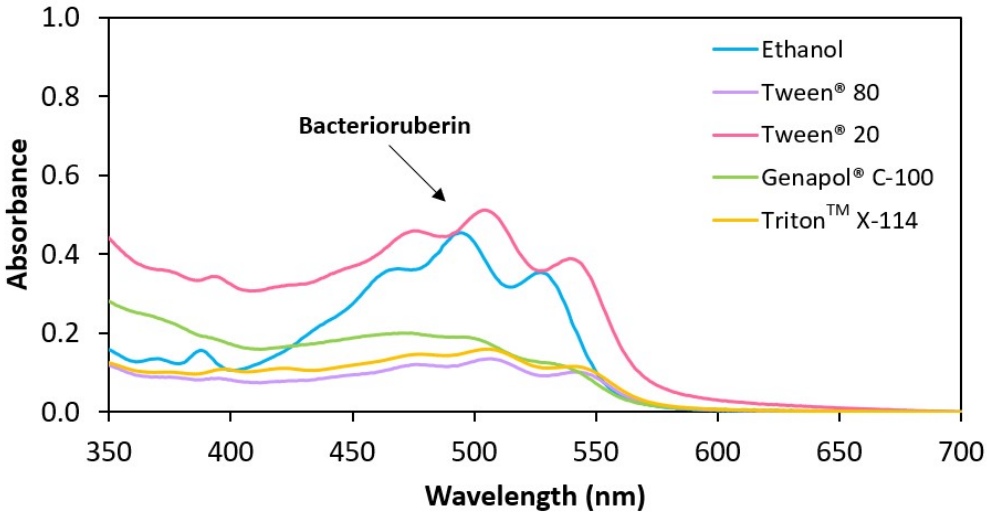


Fig. S2. UV-Vis spectroscopy of the extracts obtained in the screening of the non-ionic compounds at 250 mM.

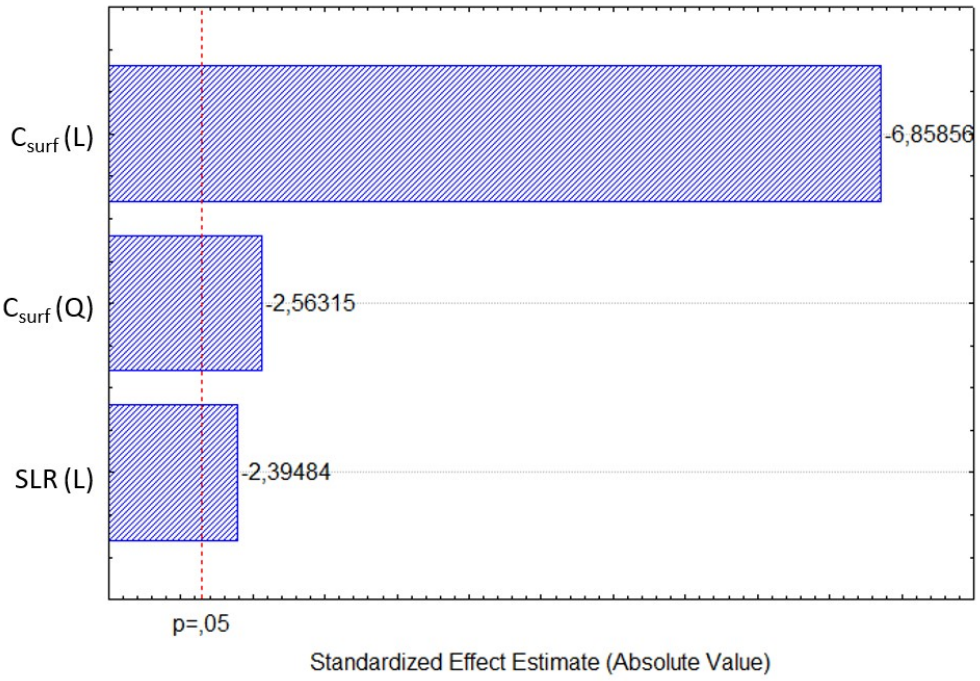


Fig. S3. Pareto Chart of the CCRD (2^3) regarding bacterioruberin yield of extraction ($mg_{bacterioruberin}.g_{wet\ biomass}^{-1}$) using aqueous solutions of Tween® 20.

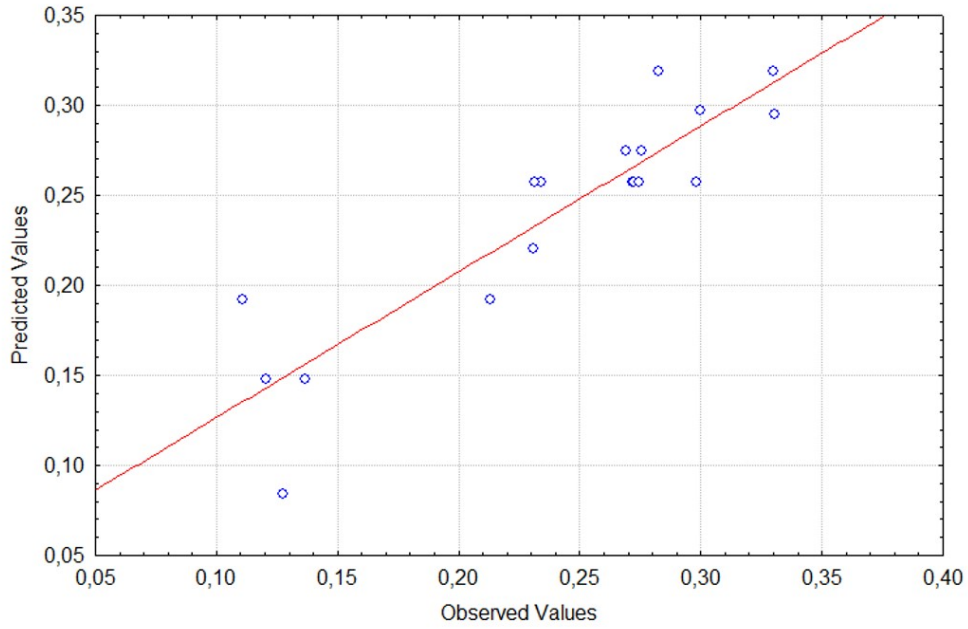


Fig. S4. Predicted *vs.* experimental values of the CCRD (2^3) regarding bacterioruberin yield of extraction ($mg_{bacterioruberin}.g_{wet\ biomass}^{-1}$) using aqueous solutions of Tween® 20.

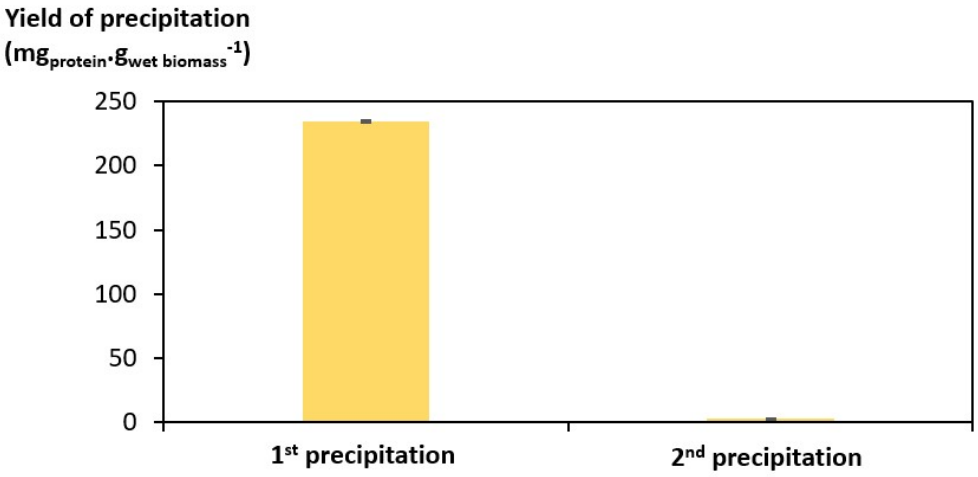


Fig. S5. Yield of precipitation ($mg_{protein}.g_{wet\ biomass}^{-1}$) for two consecutive protein precipitations using the same operational conditions, measured after proteins redissolution in PBS.

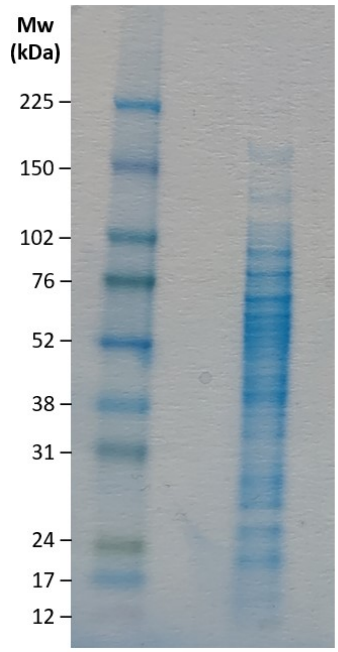


Fig. S6. SDS-PAGE of the recovered proteins redissolved in PBS after protein induced precipitation.

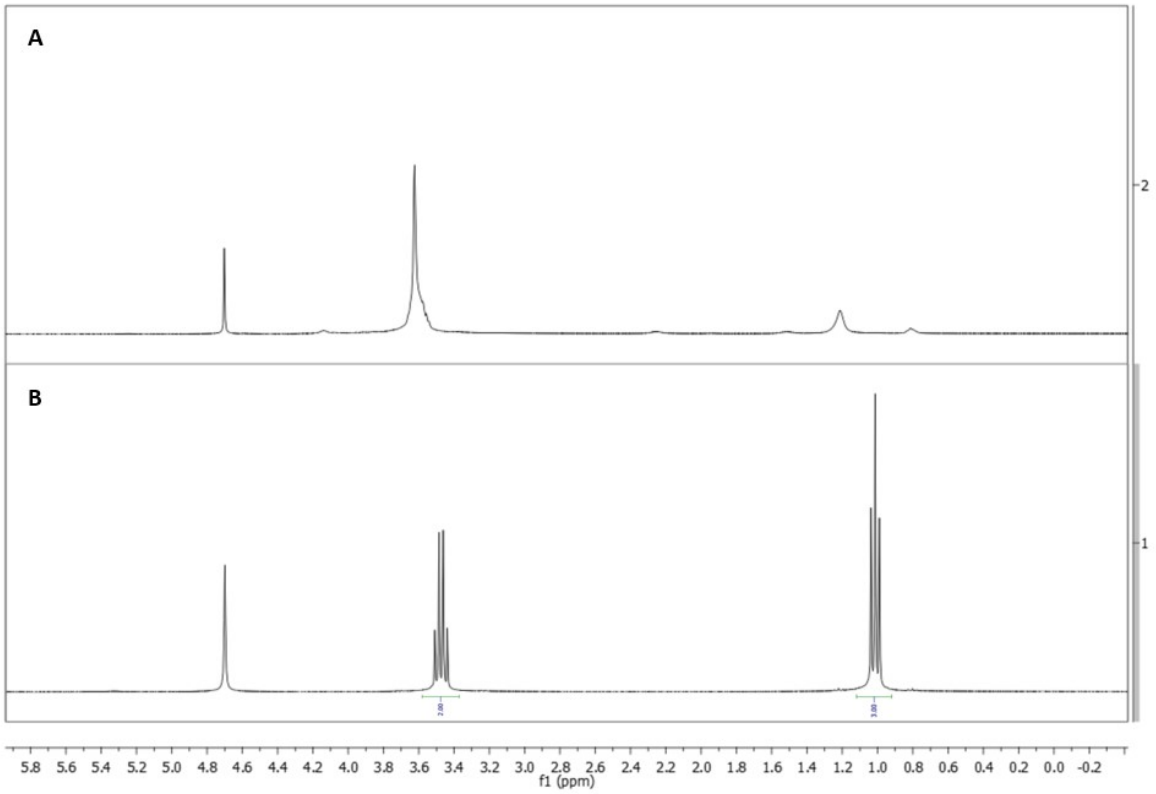


Fig. S7. ^{1}H NMR spectroscopy of (A) pure Tween® 20 and (B) ethanolic fraction rich in bacterioruberin (after the polishing step) dissolved in D_2O.

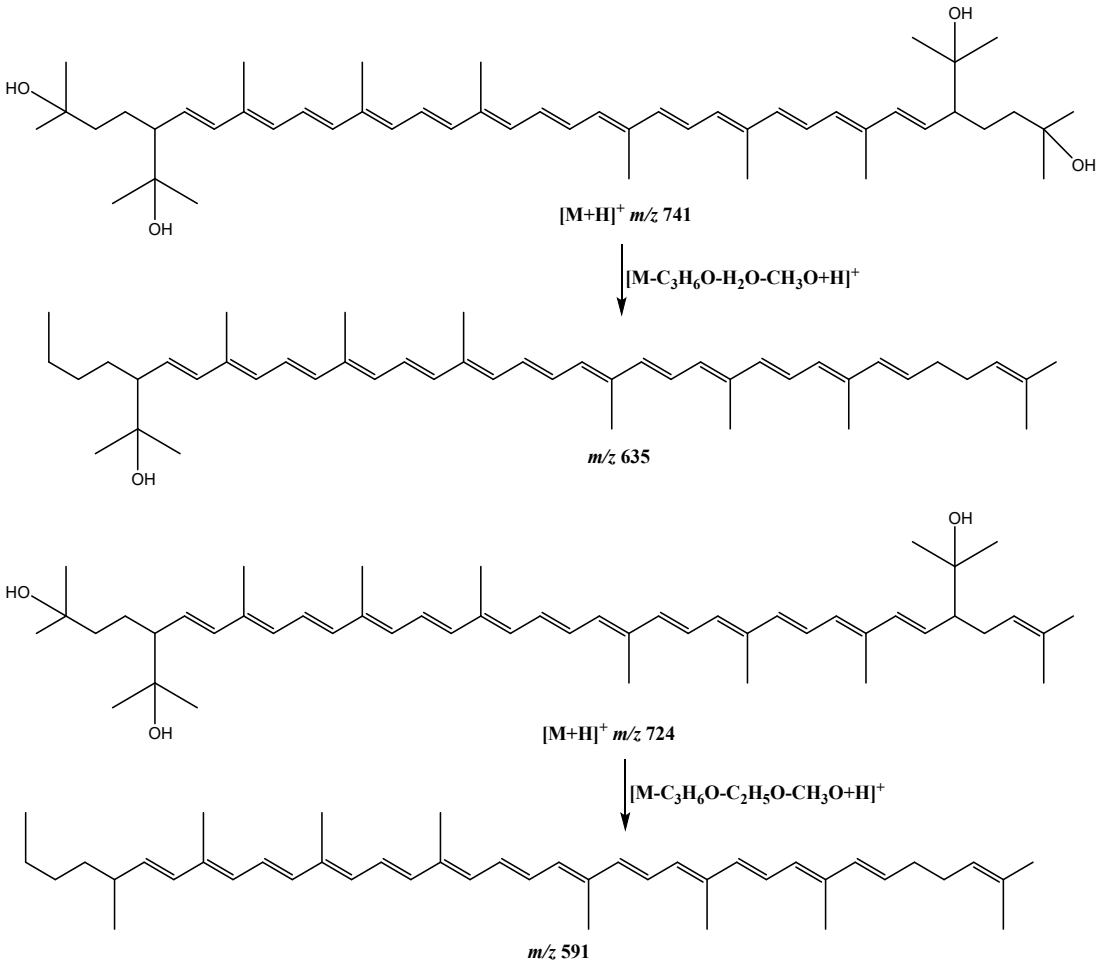
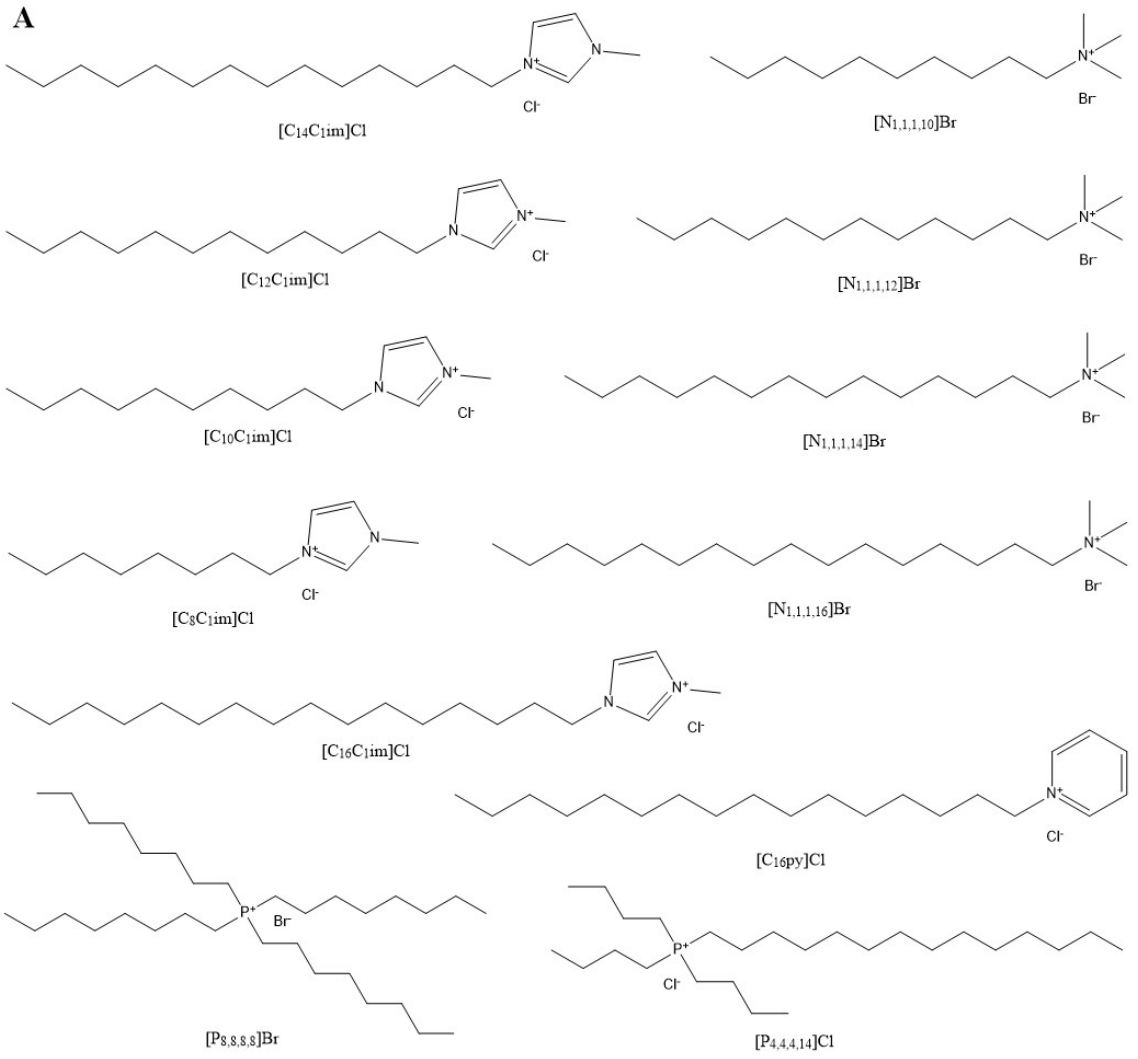


Fig. S8. Chemical structures of bacterioruberin (85 %) and monoanhydrobacterioruberin (15 %) identified by UHPLC-MS analysis.


A
[C14C1im]Cl
[N1,1,1,10]Br
[C12C1im]Cl
[N1,1,1,12]Br
[C10C1im]Cl
[N1,1,1,14]Br
[C8C1im]Cl
[N1,1,1,16]Br
[C16C1im]Cl
[C16py]Cl
[P8,8,8,8]Br
[P4,4,4,14]Cl
B
SDS
SDBS
AOT

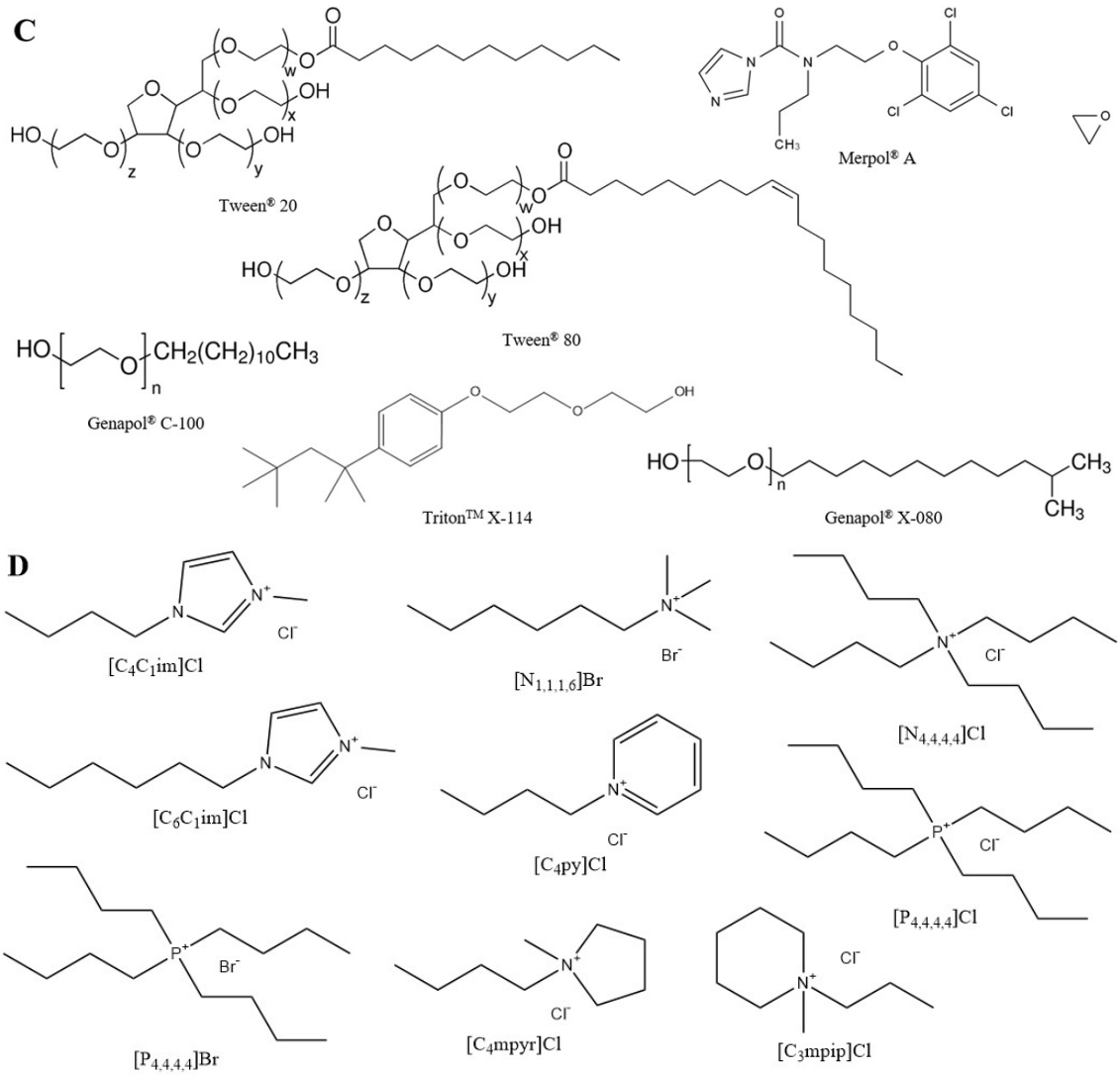


Fig. S9. Molecular structures and abbreviation names of the cationic (A), anionic (B), non-ionic (C), and non-tensioactive (D) compounds screened in this work.

Table S1. List of the surface-active compounds tested to recover bacterioruberin with the respective indication of their success or unsuccess in the extraction.

	Surface-active compound	**Success**	**Observations**
Cationic	$[C_8C_1im]Cl$	x	Formed 2 phases, inviable to read UV-Vis spectra
	$[C_{10}C_1im]Cl$	✓	White cloudy initial solution but viable to read UV-Vis spectra
	$[C_{12}C_1im]Cl$	✓	-
	$[C_{14}C_1im]Cl$	✓	-
	$[C_{16}C_1im]Cl$	✓	White cloudy initial solution but viable to read UV-Vis spectra
	$[N_{1,1,1,10}]Br$	✓	-
	$[N_{1,1,1,12}]Br$	✓	-
	$[N_{1,1,1,14}]Br$	✓	-
	$[N_{1,1,1,16}]Br$	✓	-
	$[C_{16}py]Cl$	✓	-
	$[P_{8,8,8,8}]Br$	x	Biomass did not settle in centrifuge; white cloudy initial solution
	$[P_{4,4,4,14}]Cl$	✓	-
Anionic	SDBS	x	Biomass did not settle in centrifuge
	SDS	✓	Very slimy
	AOT	x	Formed 3 phases; white cloudy initial solution;

			very slimy inviable to separate mixture
Non-ionic	Tween® 20	✓	Very slimy
	Tween® 80	✓	Very slimy
	Triton™ X-114	✓	Formed 2 phases; slimy solution but possible to separate from the sample
	Merpol® A	x	Formed 2 phases; very slimy inviable to separate mixture from sample; white cloudy initial solution
	Genapol® X-080	x	Biomass did not settle in centrifuge; white cloudy initial solution
	Genapol® C-100	✓	-
Non-tensioactive	$[C_4C_1im]Cl$	x	Biomass did not settle in centrifuge
	$[C_6C_1im]Cl$	x	Biomass did not settle in centrifuge
	$[P_{4,4,4,4}]Cl$	x	Biomass did not settle in centrifuge
	$[P_{4,4,4,4}]Br$	x	Formed 2 phases, inviable to read UV-Vis spectra
	$[N_{4,4,4,4}]Cl$	x	Biomass did not settle in centrifuge
	$[N_{1,1,1,6}]Br$	x	Biomass did not settle in centrifuge
	$[C_3mpip]Cl$	x	Biomass did not settle in centrifuge
	$[C_4mpyr]Cl$	x	Biomass did not settle in centrifuge
	$[C_4py]Cl$	x	Biomass did not settle in centrifuge

Table S2. Real values used in the optimization process by CCRD (2^3) expressed by bacterioruberin yield of extraction ($mg_{bacterioruberin} \cdot g_{wet\ biomass}^{-1}$) using aqueous solutions of Tween® 20.

Run	SLR ($g_{wet\ biomass} \cdot mL_{solvent}^{-1}$)	t (min)	C_{Surf} (mM)	Yield of extraction ($mg_{bacterioruberin} \cdot g_{wet\ biomass}^{-1}$)
1	0.118	23.0	230.0	0.330
2	0.282	23.0	230.0	0.276
3	0.118	67.0	230.0	0.283
4	0.282	67.0	230.0	0.269
5	0.118	23.0	370.0	0.111
6	0.282	23.0	370.0	0.120
7	0.118	67.0	370.0	0.213
8	0.282	67.0	370.0	0.137
9	0.0622	45.0	300.0	0.331
10	0.338	45.0	300.0	0.231
11	0.200	8.04	300.0	0.234
12	0.200	82.0	300.0	0.272
13	0.200	45.0	182.4	0.300
14	0.200	45.0	417.6	0.128
15	0.200	45.0	300.0	0.232
16	0.200	45.0	300.0	0.298
17	0.200	45.0	300.0	0.273
18	0.200	45.0	300.0	0.275

Table S3. Effect of the estimates for bacterioruberin yield of extraction ($mg_{bacterioruberin} \cdot g_{wet\ biomass}^{-1}$) using aqueous solutions of Tween® 20, optimized by the CCRD (2^3) with significant factors at 95 % confidence level.

Factor	Effect	Standard error	Calculated *t* *	p-value
Mean/Interaction	0.257	0.0107	24.1	0.000
SLR ($g_{wet\ biomass} \cdot mL_{solvent}^{-1}$) – (X1)	-0.0443	0.0185	-2.39	0.0312
C_{Surf} (mM) – (X3)	-0.127	0.0185	-6.86	0.000
C_{Surf} (mM) – ($X3^2$)	-0.0475	0.0185	-2.56	0.0225

*Degrees of freedom.

Table S4. 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 bacterioruberin yield of extraction ($mg_{bacterioruberin} \cdot g_{wet\ biomass}^{-1}$) using aqueous solutions of Tween® 20. V1, V2, and V3 represent the validation assays.

Assay	SLR ($g_{wet\ biomass} \cdot mL_{solvent}^{-1}$)	C_{Surf} (mM)	Yield of extraction ($mg_{bacterioruberin} \cdot g_{wet\ biomass}^{-1}$)		Relative deviation (%)
			Experimental values	Predicted values	
	X1	X3	Y	Predicted Y	
V1	0.06224	182.4	0.373	0.329	11.7
V2			0.361		8.84
V3			0.384		14.3
Mean of deviation					11.6

Table S5. Classification of the surface-active compounds used in this work, their respective critical micellar concentration (CMC), purity, CAS number, and supplier.

Surface-active compounds	Designation	Classification	CMC (mM) / Reference	Purity	CAS Number	Supplier
$[C_{12}C_1im]Cl$	1-dodecyl-3-methylimidazolium chloride	Cationic	15 [1]	98 wt%	114569-84-5	IoLiTec
$[C_{14}C_1im]Cl$	1-methyl-3-tetradecylimidazolium chloride	Cationic	4 [1]	98 wt%	171058-21-2	IoLiTec
$[C_{16}C_1im]Cl$	1-hexadecyl-3-methylimidazolium chloride	Cationic	1.26 [2]	>98 wt%	61546-01-8	IoLiTec
$[C_3mpip]Cl$	1-methyl-1-propylpiperidinium chloride	Non-tensioactive	-	99 wt%	1383436-85-8	IoLiTec
$[C_{16}py]Cl.H_2O$	Hexadecylpyridinium chloride monohydrate	Cationic	0.96 [3]	99.0 - 102.0 wt%	6004-24-6	Sigma-Aldrich
$[C_4C_1im]Cl$	1-butyl-3-methylimidazolium chloride	Non-tensioactive	-	99 wt%	79917-90-1	IoLiTec
$[N_{1,1,1,10}]Br$	Decyltrimethylammonium bromide	Cationic	25.2 [4]	99 wt%	2082-84-0	Tokyo Chemical Industry
$[C_4mpyr]Cl$	1-butyl-1-methylpyrrolidinium chloride	Non-tensioactive	-	99 wt%	479500-35-1	IoLiTec
$[N_{1,1,1,12}]Br$	Dodecyltrimethylammonium bromide	Cationic	14 [5]	99 wt%	1119-94-4	Alfa Aesar
$[C_4py]Cl$	1-butylpyridinium chloride	Non-tensioactive	-	98 wt%	1124-64-7	IoLiTec
$[N_{1,1,1,14}]Br$	Tetradecyltrimethylammonium bromide	Cationic	3.6 [6]	98 wt%	1119-97-7	Alfa Aesar
$[C_6C_1im]Cl$	1-hexyl-3-methylimidazolium chloride	Non-tensioactive	900 [1]	98 wt%	171058-17-6	IoLiTec
$[N_{1,1,1,16}]Br$	Hexadecyltrimethylammonium bromide	Cationic	0.98 [3]	99 wt%	57-09-0	Merk
$[C_8C_1im]Cl$	1-methyl-3-octylimidazolium chloride	Cationic	220 [1]	99 wt%	64697-40-1	IoLiTec
$[N_{1,1,1,6}]Br$	Hexyltrimethylammonium bromide	Non-tensioactive	-	98 wt%	2650-53-5	Alfa Aesar
$[C_{10}C_1im]Cl$	1-decyl-3-methylimidazolium chloride	Cationic	55 [1]	98 wt%	171058-18-7	IoLiTec

$[N_{4,4,4,4}]Cl$	Tetrabutylammonium chloride	Non-tensioactive	-	97 wt%	1112-67-0	Sigma-Aldrich
$[P_{4,4,4,14}]Cl$	Tributyltetradecylphosphonium chloride	Cationic	4.69 [4]	95 wt%	81741-28-8	IoLiTec
$[P_{4,4,4,4}]Br$	Tetrabutylphosphonium bromide	Non-tensioactive	-	95 wt%	3115-68-2	IoLiTec
$[P_{4,4,4,4}]Cl$	Tetrabutylphosphonium chloride	Non-tensioactive	-	95 wt%	2304-30-5	IoLiTec
$[P_{8,8,8,8}]Br$	Tetraoctylphosphonium bromide	Cationic	nd	-	23906-97-0	Cytec
AOT	Dioctyl sulfosuccinate sodium salt	Anionic	2.1 [7]	96 wt%	577-11-7	Sigma-Aldrich
Genapol® C-100	-	Non-ionic	0.075 [8*]	-	61791-13-7	Sigma-Aldrich
Genapol® X-080	Polyethylene glycol monoalkyl ether	Non-ionic	0.081 [9]	-	9043-30-5	Sigma-Aldrich
Merpol® A	-	Non-ionic	0.005 % [10*]	-	37208-27-8	Sigma-Aldrich

SDS	Sodium dodecylsulfate	Anionic	8 [3]	pharma grade	151-21-3	Panreac
SDBS	Sodium dodecyl-benzenesulfonate	Anionic	1.25 [3]	technical grade	25155-30-0	Sigma-Aldrich
Triton™ X-114	Polyethylene glycol *tert*-octylphenyl ether	Non-ionic	0.29 [11]	lab grade	9036-19-5	Acros Organics
Tween® 20	Polyethylene glycol sorbitan monolaurate	Non-ionic	0.078 [3]	-	9005-64-5	Acros Organics
Tween® 80	Polyethylene glycol sorbitan monooleate	Non-ionic	0.014 [3]	-	9005-65-6	Sigma-Aldrich

* Manufacturer data.

nd – not determined due to low solubility in water.

References

1 M. Blesic, M. H. Marques, N. V. Plechkova, K. R. Seddon, L. P. N. Rebelo and A. Lopes, *Green Chem.*, 2007, **9**, 481–490.

2 C. Jungnickel, J. Łuczak, J. Ranke, J. F. Fernández, A. Müller and J. Thöming, *Colloids Surfaces A Physicochem. Eng. Asp.*, 2008, **316**, 278–284.

3 Y. Shi, H. Q. Luo and N. B. Li, *Spectrochim. Acta Part A Mol. Biomol. Spectrosc.*, 2011, **78**, 1403–1407.

4 F. A. Vicente, I. S. Cardoso, T. E. Sintra, J. Lemus, E. F. Marques, S. P. M. Ventura and J. A. P. Coutinho, *J. Phys. Chem. B*, 2017, **121**, 8742–8755.

5 M. A. Bahri, M. Hoebeke, A. Grammenos, L. Delanaye, N. Vandewalle and A. Seret, *Colloids Surfaces A Physicochem. Eng. Asp.*, 2006, **290**, 206–212.

6 S. P. Moulik, M. E. Haque, P. K. Jana and A. R. Das, *J. Phys. Chem.*, 1996, **100**, 701–708.

7 J. K. Salem, I. M. El-Nahhal and S. F. Salama, *Chem. Phys. Lett.*, 2019, **730**, 445–450.

8 Merk, Genapol® C-100, https://www.sigmaaldrich.com/PT/en/product/sigma/61028, (accessed 24 October 2021).

9 E. Cordisco, C. N. Haidar, R. Goñi, B. B. Nerli and L. P. Malpiedi, *Fluid Phase Equilib.*, 2015, **393**, 111–116.

10 Stepan, MERPOL® A, https://www.stepan.com/content/dam/stepan-dot-com/webdam/website-product-documents/product-bulletins/surfactants/MERPOLA.pdf, (accessed 25 March 2022).

11 S. Wu, F. Liang, D. Hu, H. Li, W. Yang and Q. Zhu, *Anal. Chem.*, 2020, **92**, 4259–4265.