Supporting Information

Enhanced antimalarial activity of extracts of *Artemisia annua* L. achieved with aqueous solutions of salicylate salts and ionic liquids

Ana M. Ferreira,[†a] Isabela Sales,[†a,b] Sónia A. O. Santos,[a] Tiago Santos,[c] Fátima Nogueira,[c,d] Silvana Mattedi,[b] Simão P. Pinho,[e,f] João A. P. Coutinho[a] and Mara G. Freire[a]*

[a]CICECO – Aveiro Institute of Materials, Department of Chemistry, University of Aveiro, 3810-193 Aveiro, Portugal.

[b]Escola Politécnica, Universidade Federal da Bahia, Bahia, 40210-630, Brazil.

[c]Global Health and Tropical Medicine, Instituto de Higiene e Medicina Tropical, Universidade Nova de Lisboa, P-1349-008 Lisboa, Portugal.

[d]LAQV-REQUIMTE, Departamento de Química, Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa, 2829-516, Caparica, Portugal.

[e]Mountain Research Center – CIMO, Polytechnic Institute of Bragança, Portugal.

[f]SusTEC, Instituto Politécnico de Bragança, Bragança, 5300-253, Portugal.

[†]These authors contributed equally

*Corresponding author: maragfreire@ua.pt

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Synthesis of cholinium salicylate

Cholinium salicylate ([Chol][Sal]) was synthesised by the neutralisation of the base (cholinium bicarbonate, 1 mol equivalents) with salicylic acid (1.1 mol equivalents), based on standard protocol.[1] More specifically, cholinium bicarbonate was added dropwise into an aqueous solution of salicylic acid, at 0°C. The mixture was stirred overnight at room temperature, and protected from light, producing the cholinium-based IL, carbonic acid gas and water as a by-product. The excess of water was then removed under reduced pressure at 60°C. The unreacted salicylic acid accumulated in the prepared IL was eliminated by washing with ethyl acetate by liquid-liquid extraction. Finally, the obtained compound was dried under a high vacuum for at least 48 h. The structure of IL synthesised was confirmed by ^{1}H and ^{13}C NMR (*cf.* Figure S1), showing a high purity level. An uncoloured liquid was obtained at 28 °C and a total of 12 g of cholinium salicylate. The yield of the reaction was 72%.

Optimisation of the artemisinin extraction using RSM

In a 2^k response surface methodology (RSM) there are k factors that contribute to a different response, and the data are treated according to a second order polynomial equation:

$$Y = \beta_0 + \sum_i \beta_i X_i + \sum_i \beta_{ii} X_i^2 + \sum_{i<j} \beta_{ij} X_i X_j \qquad \text{(Eq. S1)}$$

where Y is the response variable and β_0, β_i, β_{ii} and β_{ij} are the adjusted coefficients for the intercept, linear, quadratic and interaction terms, respectively, and X_i and X_j are independent variables.

The design was extended up to the axial points, which are at a distance of α coded units from the central point:

$$\alpha = (2^k)^{1/4} \qquad \text{(Eq. S2)}$$

Figures

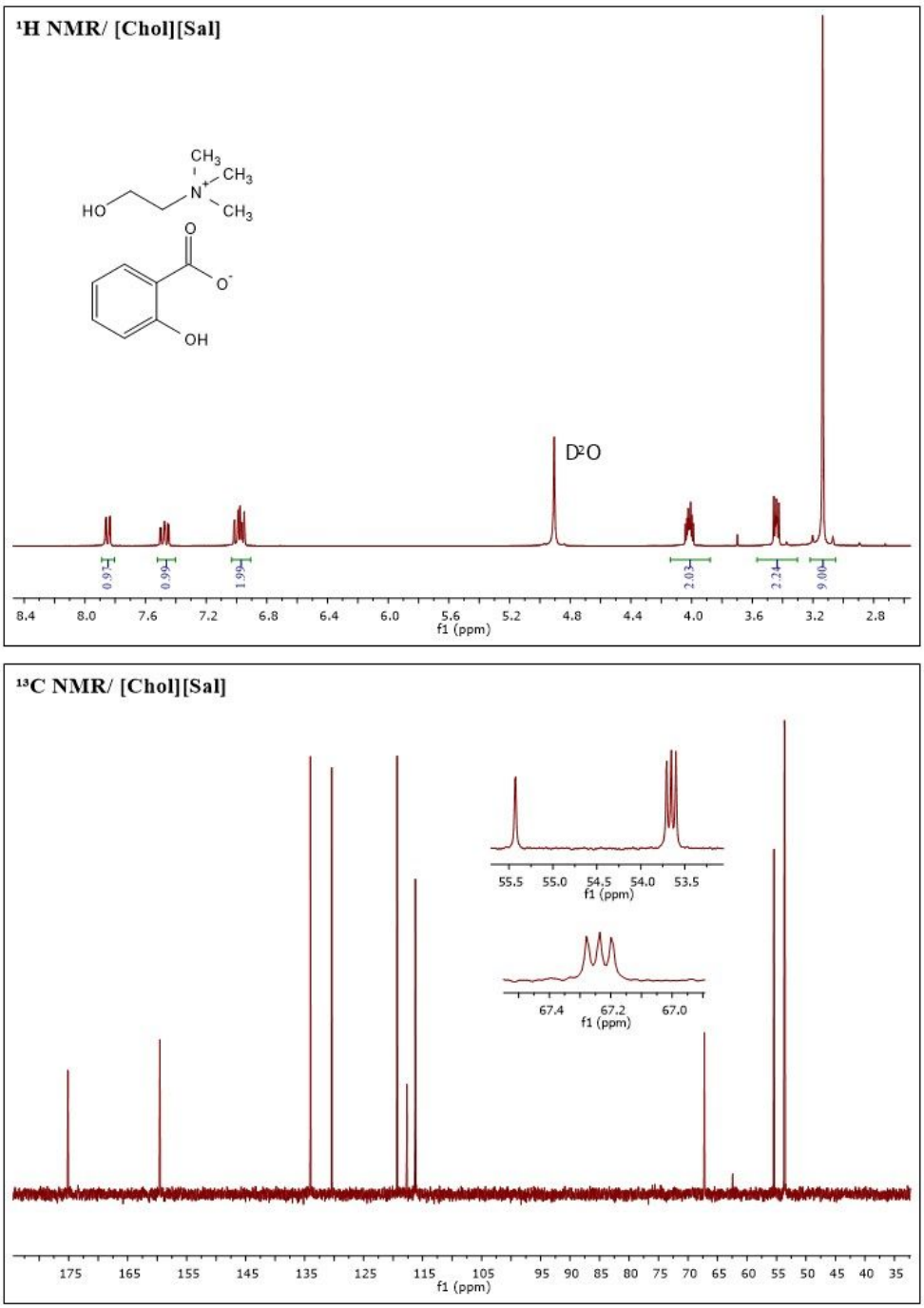

Figure S1. ^{1}H-NMR and ^{13}C-NMR of (2-Hydroxyethyl) trimethylammonium 2-hydroxybenzoate ([Chol][Sal]). ^{1}H NMR (300 MHz, D_2O): δ 7.89 – 7.80 (m, 8H), 7.47 (ddd, *J* = 8.2, 7.3, 1.8 Hz, 8H), 6.98 (tdd, *J* = 6.0, 4.9, 2.3 Hz, 16H), 4.03 (tdd, *J* = 6.8, 6.3, 3.7 Hz, 16H), 3.57 – 3.30 (m, 18H), 3.14 (s, 72H), 2.11 – 0.31 (m, 1H). ^{13}C NMR (75 MHz, D_2O) δ 175.13 (s), 159.56 (s), 134.03 (s), 130.40 (s), 119.33 (s), 117.67 (s), 116.24 (s), 67.46 – 67.06 (m), 55.43 (s), 53.89 – 53.47 (m).

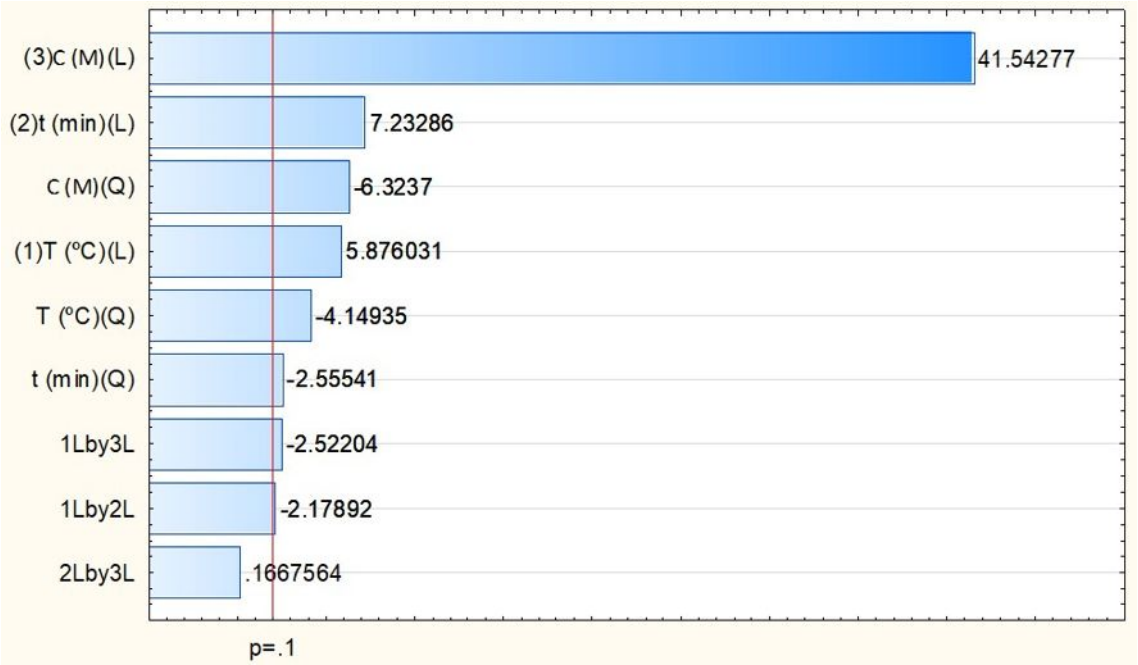

Figure S2. Pareto chart for the standardized main effects in the factorial planning for the extraction yield of artemisinin using aqueous solutions of [Chol][Sal], with 90% of confidence. The vertical line indicates the statistical significance of the several effects.

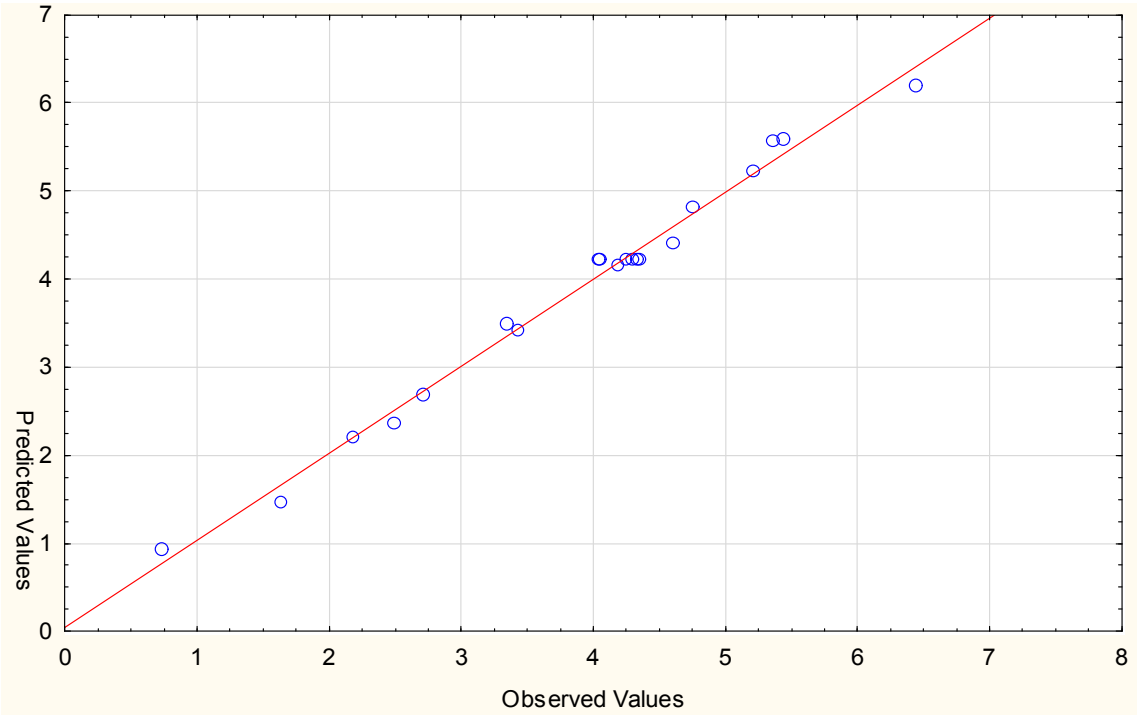

Figure S3. Predict *vs.* observed values of extraction yield of artemisinin using aqueous solutions of [Chol][Sal].

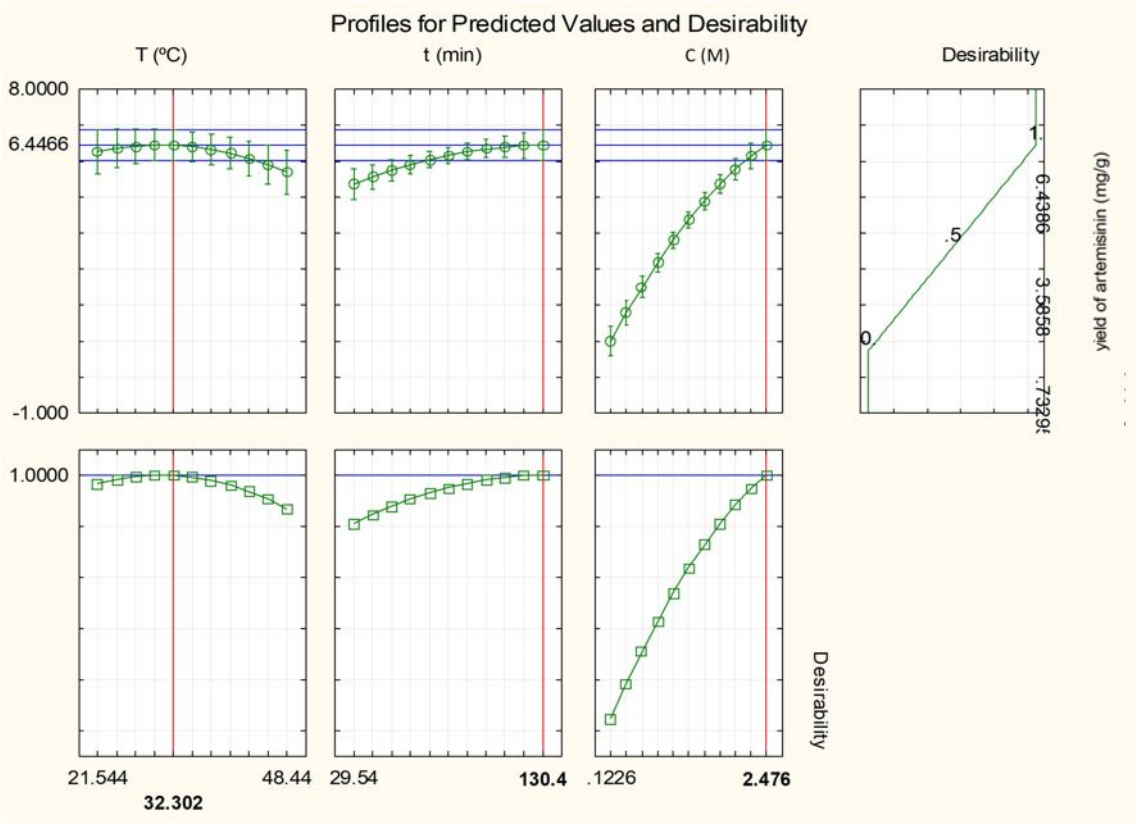

Figure S4. Profiles for predicted values and desirability function for extraction yield of artemisinin using aqueous solutions of Na[Sal] from mixture design. Red lines indicate optimized values for each variable.

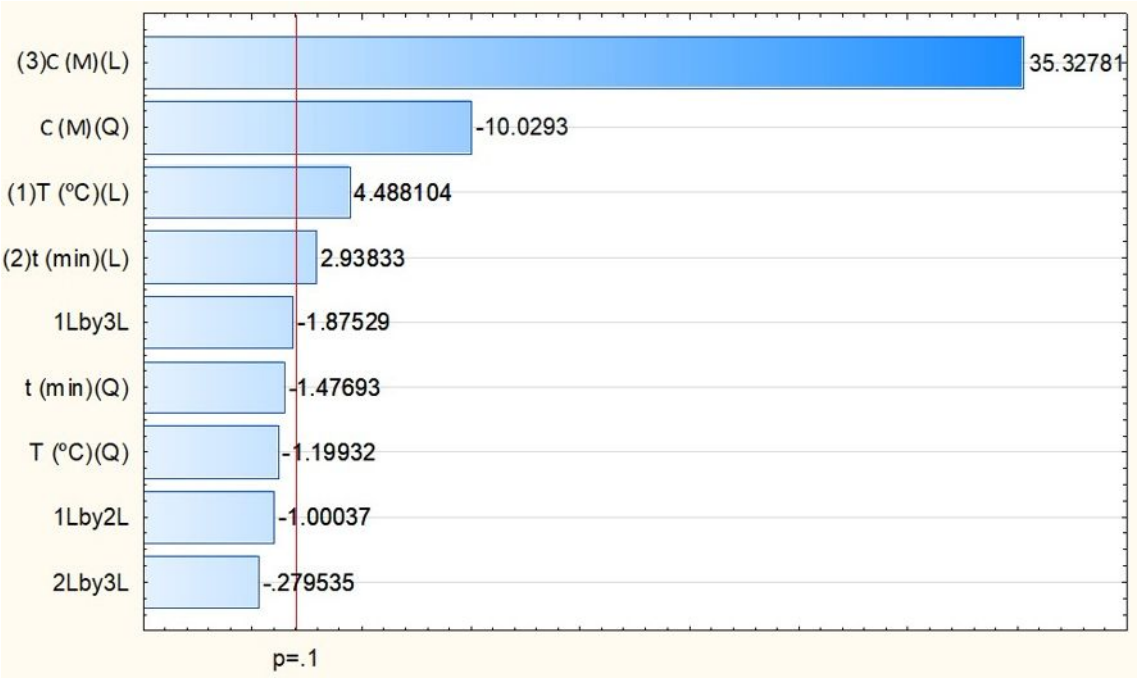

Figure S5. Pareto chart for the standardized main effects in the factorial planning for the extraction yield of artemisinin using aqueous solutions of Na[Sal], with 90% of confidence. The vertical line indicates the statistical significance of the several effects.

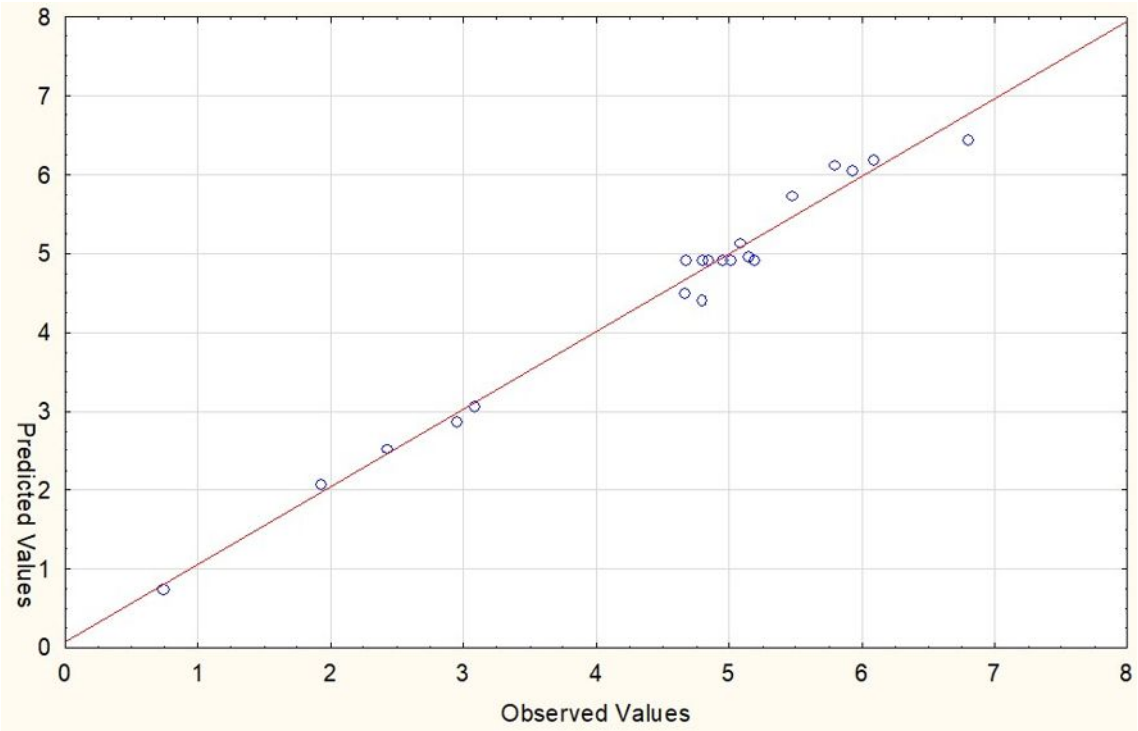

Figure S6. Predict *vs.* observed values of extraction yield of artemisinin using aqueous solutions of Na[Sal].

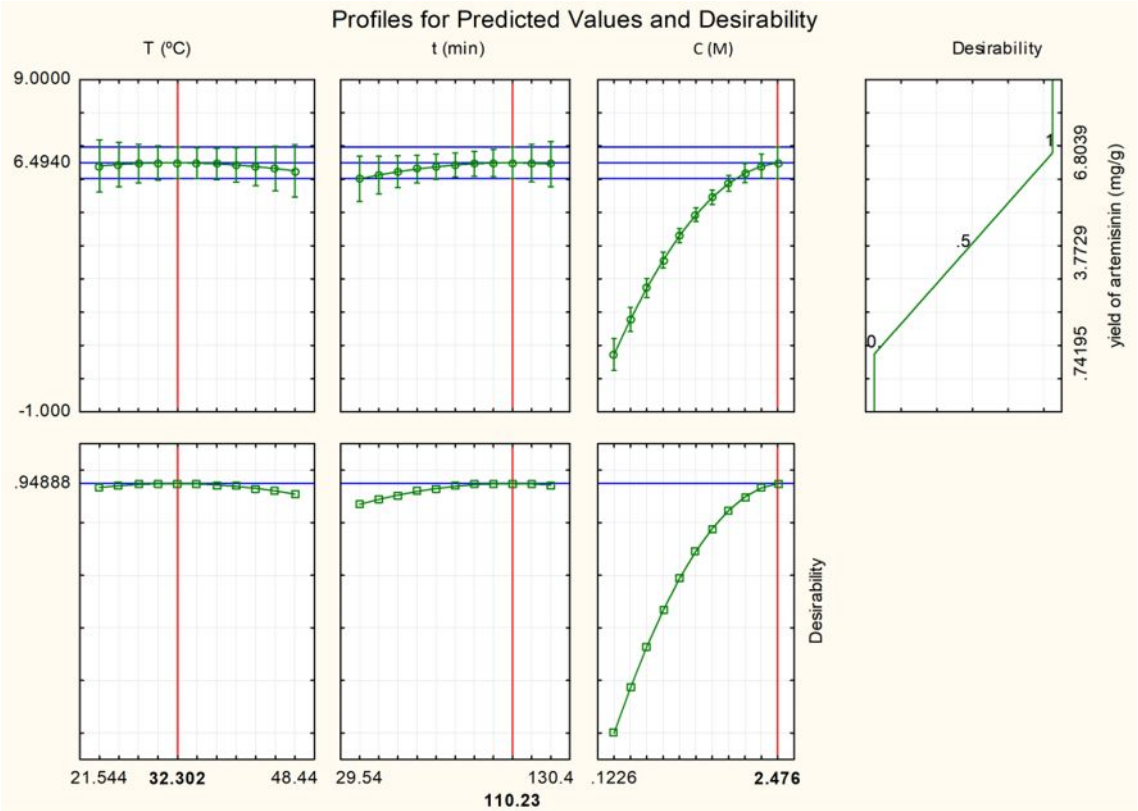

Figure S7. Profiles for predicted values and desirability function for extraction yield of artemisinin using aqueous solutions of Na[Sal] from mixture design. Red lines indicate optimized values for each variable.

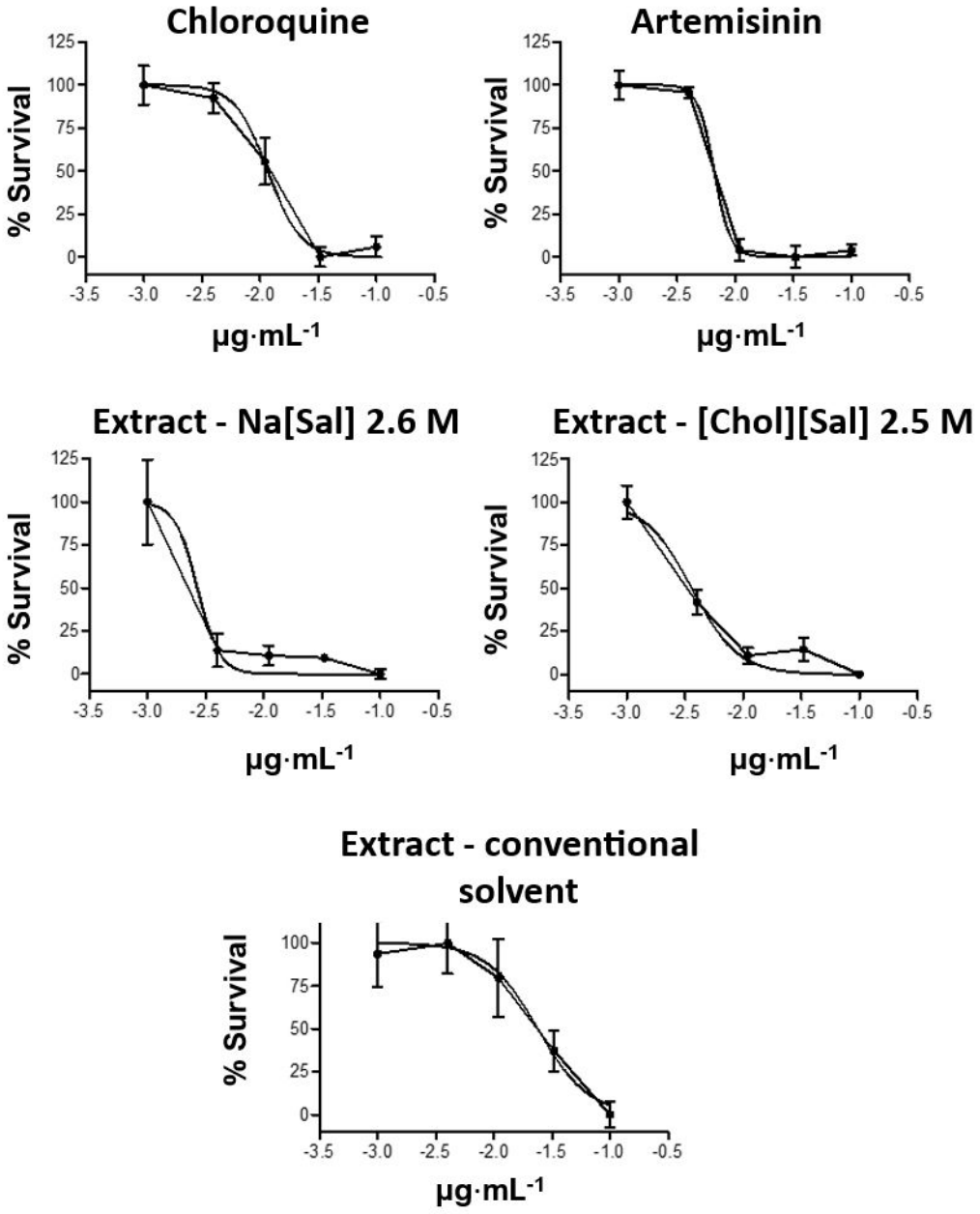

Figure S8. Representative dose response curves of *P. falciparum* inhibition in the presence of the extracts and control drugs (chloroquine and artemisinin).

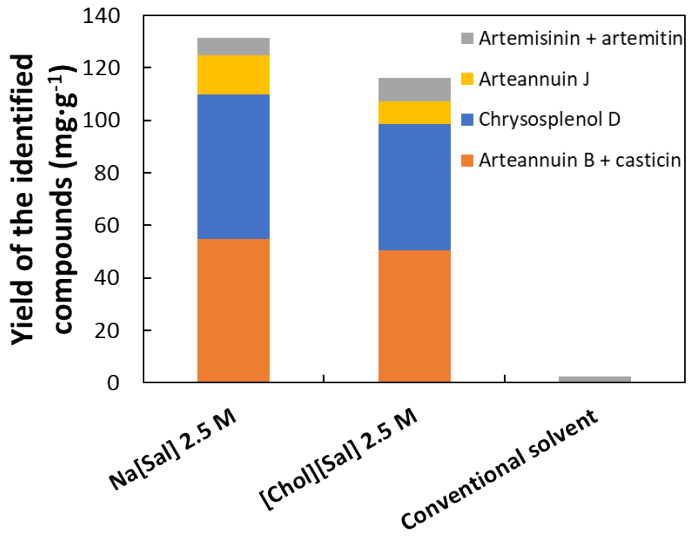

Figure S9. Yields of the identified compounds (mg·g^{-1}) in the extracts *of Artemisia Annua* L. under the different optimized conditions and using conventional solvent. Except for the extract with conventional solvent, where only artemisinin was detected.

Tables

Table S1. 2^3 factorial planning.

	X_1	X_2	X_3
1	-1	-1	-1
2	1	-1	-1
3	-1	1	-1
4	1	1	-1
5	-1	-1	1
6	1	-1	1
7	-1	1	1
8	1	1	1
9	-1.68	0	0
10	1.68	0	0
11	0	-1.68	0
12	0	1.68	0
13	0	0	-1.68
14	0	0	1.68
15	0	0	0
16	0	0	0
17	0	0	0
18	0	0	0
19	0	0	0
20	0	0	0

Table S2. Coded levels of independents variables used in the factorial planning.

	-1.682	**-1**	**0**	**1**	**1.68**
Temperature (*T*, °C)	22	27	35	43	48
Extraction time (*t*, min)	30	50	80	110	130
Concentration (*C*, mol·L^{-1})	0.12	0.60	1.30	2.00	2.48

Table S3. Artemisinin yield using aqueous solution of hydrotropes at 2 M and 3 M by SLE method and using petroleum ether by Soxhlet method. Fixed extraction conditions at SLE: S/L ratio of 1:10, 90 min and 30°C.

Salt	**C = 2 mol·L^{-1}**		**C = 3 mol·L^{-1}**	
	mg·g^{-1}	**± σ**	**mg·g^{-1}**	**± σ**
[P_{4444}]Cl	2.86	0.18	4.96	1.43
[C_4C_1im][DCA]	5.74	0.23	6.32	0.04
[C_4C_1im][SCN]	5.83	0.07	6.71	0.06
[Chol][Sal]	5.52	0.09	6.70	0.30
Na[Sal]	5.61	0.55	6.46	0.73
Conventional solvent	**mg·g^{-1}**	**± σ**		
Petroleum ether	3.76	0.20	---	---
Dichloromethane	4.65	1.12	---	---

Table S4. Experimental data and response surface predicted values of the factorial planning considering the artemisinin' extraction using aqueous solutions of [Chol][Sal].

				Extraction yield of artemisinin ($mg \cdot g^{-1}$)		
n.	***T* (°C)**	***t* (min)**	***C* ($mol \cdot L^{-1}$)**	**Observed**	**Predicted**	**Residuals**
1	27	50	0.6	1.64	1.46	0.18
2	43	50	0.6	2.49	2.37	0.12
3	27	110	0.6	2.19	2.20	-0.02
4	43	110	0.6	2.71	2.68	0.03
5	27	50	2.0	4.76	4.82	-0.06
6	43	50	2.0	5.22	5.23	-0.01
7	27	110	2.0	5.44	5.60	-0.16
8	43	110	2.0	5.36	5.58	-0.21
9	22	80	1.3	3.44	3.42	0.02
10	48	80	1.3	4.19	4.16	0.03
11	35	30	1.3	3.34	3.50	-0.15
12	35	130	1.3	4.61	4.41	0.20
13	35	80	0.1	0.73	0.93	-0.20
14	35	80	2.5	6.44	6.19	0.25
15	35	80	1.3	4.34	4.22	0.12
16	35	80	1.3	4.30	4.22	0.08
17	35	80	1.3	4.05	4.22	-0.17
18	35	80	1.3	4.03	4.22	-0.19
19	35	80	1.3	4.33	4.22	0.11
20	35	80	1.3	4.25	4.22	0.03

Table S5. Regression coefficients of the predicted second-order polynomial model for the extraction yield of artemisinin using aqueous solutions of [Chol][Sal] obtained from the RSM, $R^2 = 0.9896$; and adjusted $R^2 = 0.9802$. Note that the statist results obtained are in terms of the coded values of the factors.

	Regression coefficients	**Standard deviation**	**t-student**	**p-value**
Interception	-6.9713	1.0789	-6.4617	0.0013
(1)*T* (ºC)(L)	0.2585	0.0450	5.7401	0.0022
***T* (ºC)(Q)**	-0.0024	0.0006	-4.1493	0.0089
(2)*t* (min)(L)	0.0408	0.0102	3.9916	0.0104
***t* (min)(Q)**	-0.0001	0.0000	-2.5554	0.0509
(3)*C* (M)(L)	4.2072	0.4125	10.1983	0.0002
***C* (M)(Q)**	-0.4731	0.0748	-6.3237	0.0015
1L by 2L	-0.0004	0.0002	-2.1789	0.0812
1L by 3L	-0.0221	0.0088	-2.5220	0.0530
2L by 3L	0.0004	0.0023	0.1668	0.8741

Table S6. ANOVA data for the extraction yield of artemisinin using aqueous solutions of [Chol][Sal] obtained from the RSM design.

	Sum of squares	Degrees of freedom	Mean square	F-value	p-value
Regression	36.344	9	4.038	105.469	1.02E-08
Residuals	0.383	10	0.038		
Total	36.727				

Table S7. Experimental data and response surface predicted values of the factorial planning considering the artemisinin' extraction using aqueous solutions of Na[Sal].

				Extraction yield of artemisinin ($mg \cdot g^{-1}$)		
n.	***T* (°C)**	***t* (min)**	***C* ($mol \cdot L^{-1}$)**	**Observed**	**Predicted**	**Residuals**
1	27	50	0.60	1.93	2.09	-0.16
2	43	50	0.60	2.95	2.87	0.07
3	27	110	0.60	2.42	2.53	-0.11
4	43	110	0.60	3.08	3.07	0.02
5	27	50	2.00	5.47	5.74	-0.26
6	43	50	2.00	5.92	6.06	-0.14
7	27	110	2.00	5.79	6.11	-0.32
8	43	110	2.00	6.09	6.18	-0.09
9	22	80	1.30	4.79	4.40	0.39
10	48	80	1.30	5.09	5.12	-0.04
11	35	30	1.30	4.66	4.49	0.17
12	35	130	1.30	5.14	4.96	0.18
13	35	80	0.12	0.74	0.75	-0.01
14	35	80	2.48	6.80	6.44	0.36
15	35	80	1.30	4.84	4.92	-0.08
16	35	80	1.30	5.18	4.92	0.26
17	35	80	1.30	4.67	4.92	-0.25
18	35	80	1.30	5.01	4.92	0.09
19	35	80	1.30	4.95	4.92	0.03
20	35	80	1.30	4.81	4.92	-0.11

Table S8. Regression coefficients of the predicted second-order polynomial model for the extraction yield of artemisinin using aqueous solutions of Na[Sal] obtained from the RSM, $R^2 = 0.98318$; and adjusted $R^2 = 0.96804$. Note that the statist results obtained are in terms of the coded values of the factors.

	Regression coefficients	**Standard deviation**	**t-student**	**p-value**
Interception	-4.4761	1.3723	-3.2618	0.0224
(1)*T* (ºC)(L)	0.1361	0.0573	2.3759	0.0635
***T* (ºC)(Q)**	-0.0009	0.0007	-1.1993	0.2841
(2)*t* (min)(L)	0.0271	0.0130	2.0851	0.0915
***t* (min)(Q)**	-0.0001	0.0001	-1.4769	0.1997
(3)*C* (M)(L)	5.6966	0.5247	10.8563	0.0001
***C* (M)(Q)**	-0.9543	0.0952	-10.0293	0.0002
1L by 2L	-0.0003	0.0003	-1.0004	0.3631
1L by 3L	-0.0209	0.0112	-1.8753	0.1196
2L by 3L	-0.0008	0.0030	-0.2795	0.7910

Table S9. ANOVA data for the extraction yield of artemisinin using aqueous solutions of Na[Sal] obtained from the RSM design.

	Sum of squares	**Degrees of freedom**	**Mean square**	**F-value**	**p-value**
Regression	43.266	9	4.807	64.939	1.10E-07
Residuals	0.740	10	0.074		
Total	44.006				

Table S10. IC_{50} of extracts obtained with aqueous solutions of hydrotropes.

	IC_{50} (µg·mL^{-1})
	Mean ± σ
Artemisinin	0.0066 ± 0.0025
Chloroquine	0.0099 ± 0.0016
Extract in Na[Sal] 2.5 M	0.0027 ± 0.0005
Extract in [Chol][Sal] 2.5 M	0.0034 ± 0.0009
Extract in conventional solvent (Dichloromethane)	0.0200 ± 0.0078
Na[Sal] 2.5 M	N/A
[Chol][Sal] 2.5 M	N/A

Table S11. *In vitro* haemolysis activity of the extracts obtained with aqueous solutions of hydrotropes on uninfected erythrocytes.

	Mean Haemolysis % ± SD						
	10 µg·ml^{-1}	**2 µg·ml^{-1}**	**0.4 µg·ml^{-1}**	**0.08 µg·ml^{-1}**	**0.016 µg·ml^{-1}**	**0.003 µg·ml^{-1}**	**0.001 µg·ml^{-1}**
Artemisinin	1.28 ± 0.99	0.56 ± 0.26	0.78 ± 0.11	0.62 ± 0.11	0.78 ± 0,06	0.82 ± 0.09	0.86 ± 0,09
Chloroquine	0.78 ± 0.34	0.44 ± 0.21	0..35 ± 0.26	0.34 ± 0.29	0.33 ± 0.13	0.25 ± 0.23	0.17 ± 0.08
Extract in Na[Sal] 2.5 M	30.90 ± 3.16	5.95 ± 0.33	1.03 ± 0.22	0.26 ± 0.05	NA	NA	NA
Extract in Chol[Sal] 2.5 M	58.51 ± 2.93	3.72 ± 0.35	0.97 ± 0.11	0.21 ± 0.06	NA	0.15 ± 0.09	NA
Extract in conventional solvent (Dichloromethane)	0.22 ± 0.15	0.16 ± 0.06	0.74 ± 0.14	0.02 ± 0.03	NA	NA	0.04 ± 0.10
[Na][Sal] 2.5 M	0.67 ± 0.15	0.63 ± 0.17	NA	NA	0.01 ± 0.03	0.04 ± 0.10	0.26 ± 0.19
[Chol][Sal] 2.5 M	0.54 ± 0.29	0.53 ± 0.28	0.46 ± 0.12	0.26 ± 0.16	NA	NA	NA

Table S12. UHPLC-DAD-MS^n data of compounds identified in the *Artemisia Annua* L. extracts. m/z: relative intensity.

Compound n.	Rt (min)	λ (nm)	$[M+H]^+$	Product ions (*m/z*)	Tentative identification	Reference
1	13.8	212, 258, 349	361	346(60), 328(100), 311(20)	Chrysosplenol D	[2]
2	14.6	213, 257, 347	249	231(100), 221(20), 213(20)	Arteannuin B	[3]
3	14.7	213, 257, 347	375	360(60), 342(100), 317(20)	Casticin	[2]
4	15.4	221	283	265(100), 247(70), 219(50), 201(50)	Artemisinin	Standard
5	15.5	221	389	374(40), 359(50), 356(100)	Artemitin	[4]
6	16.4	226	235	217(100), 189(20), 161(80)	Arteannuin J	[5]

Table S13. Abundance ($mg \cdot g^{-1}$) of compounds detected in the *Artemisia Annua* L. extracts.

Compound	Na[Sal] 2.5 M	[Chol][Sal] 2.5 M	Conventional Solvent (Dichloromethane)
Chrysosplenol D	54.70 ± 3.74	48.05 ± 2.92	---
Arteannuin B + casticin	54.98 ± 3.08	50.61 ± 3.54	---
Artemisinin + artemitin[a]	6.66 ± 0.27	8.85 ± 0.45	2.40 ± 0.30
Arteannuin J	14.90 ± 1.19	8.51 ± 0.43	---
Total	131.24	116.02	2.40

[a]except for conventional solvent extract, for which only artemisinin was detected.

Chemical characterization of the optimized extracts by UHPLC-DAD-MS^n

- Chrysosplenol D was identified based on its molecular ion $[M + H]^+$ at *m/z* 361, and its MS^2 spectrum, which showed the product ions at *m/z* 346 $[M + H–CH_3]^+$ and 328 $[M + H–CH_3–H_2O]^+$, characteristic of this compound.[2]
- Compound 2 was identified as arteannuin B, based on its molecular ion $[M + H]^+$ at *m/z* 249 and the respective MS^2 fragmentation, which generated the ions at *m/z* 231 $[M + H–H_2O]^+$, 221$[M + H–CO]^+$ and 213 $[M + H–2H_2O]^+$.[3]
- Casticin was identified based on its molecular ion $[M + H]^+$ at *m/z* 375 which generated upon fragmentation the product ions at *m/z* 360 $[M + H–CH_3]^+$, 342 $[M + H–CH_3–H_2O]^+$ and 317 $[M + H–2CH_3–CO]^+$.[2]
- Compound 4 was identified as artemisinin based on its retention time, and MS data, namely the molecular ion $[M + H]^+$ at *m/z* 283 and its fragmentation profile (Table S12) which are concordant with the respective standard.
- Based on the molecular ion $[M + H]^+$ at *m/z* 389 and its product ions at *m/z* 374 $[M + H–CH_3]^+$, 359 $[M + H–CH_3–CH_3]^+$, 356(100) $[M + H–CH_3–H_2O]^+$, compound 5 was identified as artemitin.[4]
- Compound 6 was identified as arteannuin J, based on the molecular ion $[M + H]^+$ at *m/z* 235, which generated upon MS^2 fragmentation the product ions at *m/z* 217 $[M + H–H_2O]^+$, 189 $[M + H–H_2O–CO]^+$, 161$[M + H–H_2O–2CO]^+$.[5]

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