

Supplementary Material for

Terpenes and Cannabinoids in Supercritical CO₂ Extracts of Industrial Hemp Inflorescences: Optimization of Extraction, Antiradical and Antibacterial Activity

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Content of listed material

Table S1. Analysis of variance (ANOVA) for the response surface quadratic models for extraction yield.

Source	Sum of squares	df	Mean square	F-value	<i>p</i> -value*
<i>Extraction yield</i>					
Model	60.50	5	12.10	104.76	< 0.0001
X ₁	54.33	1	54.33	470.38	< 0.0001
X ₂	0.9855	1	0.9855	8.53	0.0223
X ₁ ²	3.15	1	3.15	27.31	0.0012
X ₂ ²	1.77	1	1.77	15.32	0.0058
X ₁ X ₂	0.1119	1	0.1119	0.9685	0.3578
Residual	0.8085	7	0.1155		
Lack of fit	0.6647	3	0.2216	6.16	0.0557
Pure error	0.1439	4	0.0360		
Cor total	61.31	12			
R ² =0.9868					

* X₁: extraction pressure; X₂: temperature

p ≥ 0.05 not significant.

Table S2. Analysis of variance (ANOVA) for the response surface quadratic models for selected terpenes.

Source	Sum of squares	df	Mean square	F-value	<i>p</i> -value*
<i>α-Pinene</i>					
Model	51.80	5	10.36	9.47	0.0051
X ₁	30.64	1	30.64	28.00	0.0011
X ₂	0.9380	1	0.9380	0.8573	0.3853
X ₁ ²	1.65	1	1.65	1.51	0.2589
X ₂ ²	18.45	1	18.45	16.87	0.0045
X ₁ X ₂	0.8101	1	0.8101	0.7405	0.4180
Residual	7.66	7	1.09		
Lack of fit	6.12	3	2.04	5.32	0.0701
Pure error	1.54	4	0.3839		
Cor total	59.46	12			
R ² =0.8712					
<i>β-Pinene</i>					
Model	22.95	5	4.59	18.42	0.0007
X ₁	15.44	1	15.44	61.96	0.0001
X ₂	0.1507	1	0.1507	0.6045	0.4623
X ₁ ²	0.3782	1	0.3782	1.52	0.2578
X ₂ ²	6.94	1	6.94	27.86	0.0012
X ₁ X ₂	0.0222	1	0.0222	0.0891	0.7740
Residual	1.74	7	0.2492		
Lack of fit	1.25	3	0.4178	3.40	0.1338
Pure error	0.4911	4	0.1228		
Cor total	24.70	12			
R ² =0.9294					
<i>β-Myrcene</i>					
Model	220.57	5	44.11	4.27	0.0421
X ₁	110.96	1	110.96	10.75	0.0135
X ₂	0.2220	1	0.2220	0.0215	0.8875
X ₁ ²	22.37	1	22.37	2.17	0.1845
X ₂ ²	87.01	1	87.01	8.43	0.0229
X ₁ X ₂	1.50	1	1.50	0.1452	0.7145
Residual	72.27	7	10.32		
Lack of fit	54.63	3	18.21	4.13	0.1022
Pure error	17.64	4	4.41		
Cor total	292.84	12			
R ² =0.7532					
<i>Limonene</i>					
Model	16.12	5	3.22	19.88	0.0005
X ₁	12.02	1	12.02	74.12	< 0.0001
X ₂	0.0438	1	0.0438	0.2701	0.6193
X ₁ ²	0.4160	1	0.4160	2.56	0.1533
X ₂ ²	3.59	1	3.59	22.13	0.0022
X ₁ X ₂	0.0005	1	0.0005	0.0030	0.9578
Residual	1.14	7	0.1622		

Lack of fit	0.4926	3	0.1642	1.02	0.4716
Pure error	0.6429	4	0.1607		
Cor total	17.26	12			
R ² =0.9342					
<i>β -Caryophyllene</i>					
Model	41.60	5	8.32	6.02	0.0178
X ₁	6.11	1	6.11	4.42	0.0736
X ₂	5.98	1	5.98	4.33	0.0759
X ₁ ²	13.88	1	13.88	10.04	0.0157
X ₂ ²	15.62	1	15.62	11.31	0.0120
X ₁ X ₂	0.1693	1	0.1693	0.1226	0.7366
Residual	9.67	7	1.38		
Lack of fit	6.56	3	2.19	2.81	0.1721
Pure error	3.11	4	0.7785		
Cor total	51.27	12			
R ² =0.8114					
<i>Guaiol</i>					
Model	14.69	5	2.94	8.57	0.0068
X ₁	11.16	1	11.16	32.55	0.0007
X ₂	0.0197	1	0.0197	0.0576	0.8172
X ₁ ²	0.1190	1	0.1190	0.3472	0.5742
X ₂ ²	2.82	1	2.82	8.22	0.0241
X ₁ X ₂	0.9472	1	0.9472	2.76	0.1404
Residual	2.40	7	0.3428		
Lack of fit	1.96	3	0.6547	6.01	0.0579
Pure error	0.4355	4	0.1089		
Cor total	17.09	12			
R ² =0.8596					
<i>γ-Eudesmol</i>					
Model	15.69	5	3.14	6.29	0.0159
X ₁	12.08	1	12.08	24.23	0.0017
X ₂	0.0676	1	0.0676	0.1356	0.7236
X ₁ ²	0.1332	1	0.1332	0.2671	0.6212
X ₂ ²	2.59	1	2.59	5.19	0.0567
X ₁ X ₂	1.23	1	1.23	2.46	0.1606
Residual	3.49	7	0.4987		
Lack of fit	2.85	3	0.9499	5.93	0.0592
Pure error	0.6411	4	0.1603		
Cor total	19.18	12			
R ² =0.8180					
<i>Bulnesol</i>					
Model	10.49	5	2.10	5.76	0.0201
X ₁	9.15	1	9.15	25.11	0.0015
X ₂	0.0067	1	0.0067	0.0184	0.8959
X ₁ ²	0.1296	1	0.1296	0.3556	0.5697
X ₂ ²	0.7073	1	0.7073	1.94	0.2062
X ₁ X ₂	0.6530	1	0.6530	1.79	0.2225

Residual	2.55	7	0.3644		
Lack of fit	2.05	3	0.6836	5.47	0.0672
Pure error	0.5001	4	0.1250		
Cor total	13.04	12			
R ² =0.8044					

* X₁: extraction pressure; X₂: temperature
 $p \geq 0.05$ not significant.

Table S3. Analysis of variance (ANOVA) for the response surface quadratic models for CBD and CBDA.

Source	Sum of squares	df	Mean square	F-value	<i>p</i> -value*
<i>CBD</i>					
Model	27.07	5	5.41	17.00	0.0009
X ₁	2.48	1	2.48	7.79	0.0269
X ₂	10.66	1	10.66	33.48	0.0007
X ₁ ²	0.3306	1	0.3306	1.04	0.3422
X ₂ ²	12.36	1	12.36	38.81	0.0004
X ₁ X ₂	0.4159	1	0.4159	1.31	0.2907
Residual	2.23	7	0.3185		
Lack of fit	1.68	3	0.5609	4.10	0.1031
Pure error	0.5467	4	0.1367		
Cor total	29.30	12			
R ² =0.9239					
<i>CBDA</i>					
Model	762.17	5	152.43	8.37	0.0072
X ₁	648.40	1	648.40	35.59	0.0006
X ₂	18.66	1	18.66	1.02	0.3452
X ₁ ²	4.75	1	4.75	0.2609	0.6252
X ₂ ²	66.76	1	66.76	3.66	0.0971
X ₁ X ₂	34.61	1	34.61	1.90	0.2106
Residual	127.52	7	18.22		
Lack of fit	80.55	3	26.85	2.29	0.2206
Pure error	46.97	4	11.74		
Cor total	889.69	12			
R ² =0.8567					

Table S4. Analysis of variance (ANOVA) for the response surface quadratic models for antioxidant and antibacterial activity.

Source	Sum of squares	df	Mean square	F-value	<i>p</i> -value*
<i>DPPH</i>					
Model	134.67	5	26.93	5.93	0.0185
X ₁	81.11	1	81.11	17.87	0.0039
X ₂	0.6904	1	0.6904	0.1521	0.7081
X ₁ ²	0.6241	1	0.6241	0.1375	0.7217
X ₂ ²	51.99	1	51.99	11.45	0.0117
X ₁ X ₂	2.09	1	2.09	0.4616	0.5187
Residual	31.77	7	4.54		
Lack of fit	25.80	3	8.60	5.77	0.0619
Pure error	5.97	4	1.49		
Cor total	166.44	12			
R ² =0.8091					
<i>MIC (B. subtilis)</i>					
Model	620.02	5	124.00	5.05	0.0280
X ₁	188.85	1	188.85	7.70	0.0275
X ₂	209.24	1	209.24	8.53	0.0223
X ₁ ²	68.56	1	68.56	2.79	0.1385
X ₂ ²	4.25	1	4.25	0.1732	0.6898
X ₁ X ₂	140.15	1	140.15	5.71	0.0482
Residual	171.75	7	24.54		
Lack of fit	31.65	3	10.55	0.3012	0.8241
Pure error	140.10	4	35.03		
Cor total	791.78	12			
R ² =0.7831					

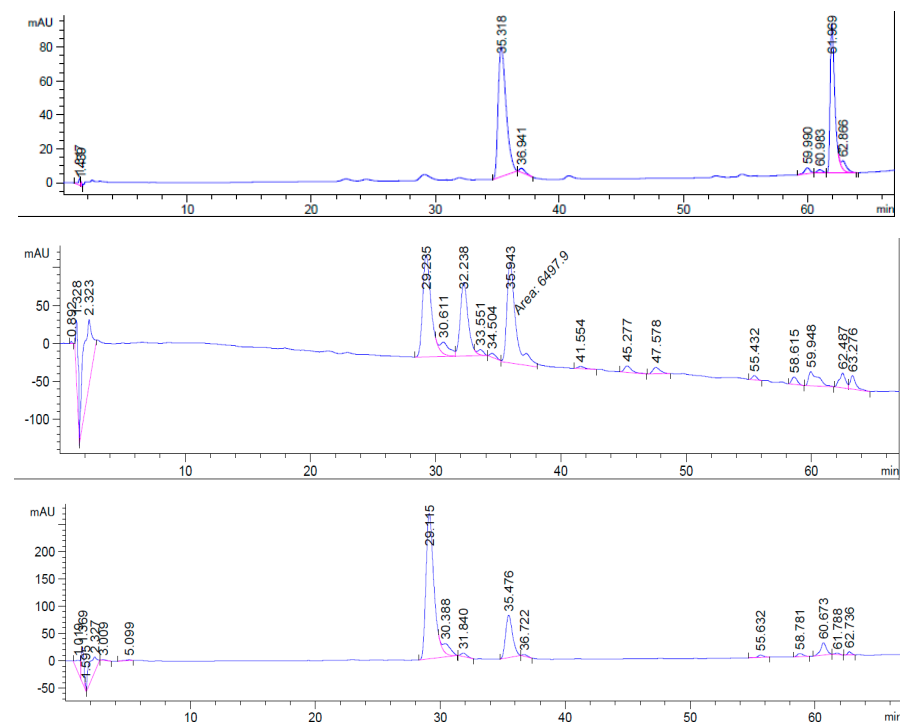


Figure S1. Representative chromatogram of the HPLC analysis of the extracts number 4; upper chromatogram at 250 nm – CBCA at 62.735 min; mean chromatogram at 210 nm – CBG at 33.243 min, THC at 47.578 min; lower chromatogram at 230 nm – CBD at 31.840 min, CBDA at 35.476 min, CBC at 55.632 min and THCA at 60.673 min.