Article

Variation in the Content of Bioactive Compounds in Infusions Prepared from Different Parts of Wild Polish Stinging Nettle (*Urtica Dioica* L.)

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Table S1. ANOVA for Response Surface Linear Model for fresh nettle leaves for the DPPH method.

Source	Sum of Squares	df	Mean Square	F Value	*p*-Value Prob > F	
Model	3.67	1	3.67	30.44	0.0002	significant
B-Temperature	3.67	1	3.67	30.44	0.0002	
Residual	1.33	11	0.12			
Lack of Fit	1.04	7	0.15	2.04	0.2557	not significant
Pure Error	0.29	4	0.072			
Cor Total	4.99	12				

Std. Dev.	0.35	R-Squared	0.7346
Mean	1.90	Adj R-Squared	0.7104
C.V. %	18.30	Pred R-Squared	0.6210
PRESS	1.89	Adeq Precision	13.957

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	1.88	1	0.096	1.66	2.09	
B-Temperature	0.67	1	0.12	0.40	0.94	1.00

Table S2. ANOVA for Response Surface Linear Model for fresh nettle leaves for the Folin-Ciocalteu method.

Source	**Sum of Squares**	**df**	**Mean Square**	**F Value**	***p*-Value Prob > F**	
Model	2.33	1	2.33	180.59	< 0.0001	significant
B-Temperature	2.33	1	2.33	180.59	< 0.0001	
Residual	0.14	11	0.013			
Lack of Fit	0.13	7	0.019	5.91	0.0527	not significant
Pure Error	0.013	4	3.135E-003			
Cor Total	2.48	12				

Std. Dev.	0.11	R-Squared	0.9426	
Mean	1.74	Adj R-Squared	0.9374	
C.V. %	6.52	Pred R-Squared	0.9252	
PRESS	0.19	Adeq Precision	33.995	

Factor	Coefficient Estimate	df	Standard Error	95% CI Low	95% CI High	VIF
Intercept	1.73	1	0.032	1.66	1.80	
B-Temperature	0.54	1	0.040	0.45	0.62	1.00

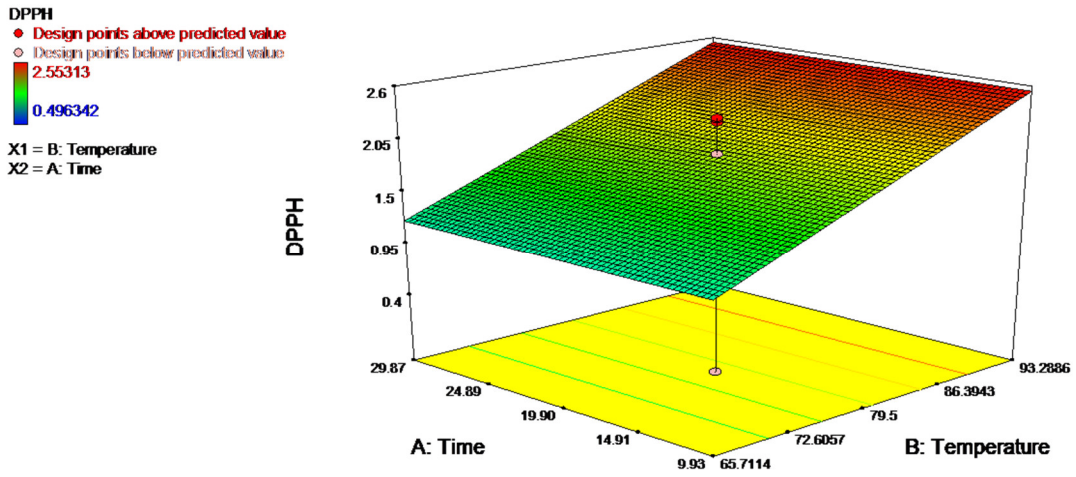


Figure S1. Model graph for fresh nettle obtained for the DPPH method.

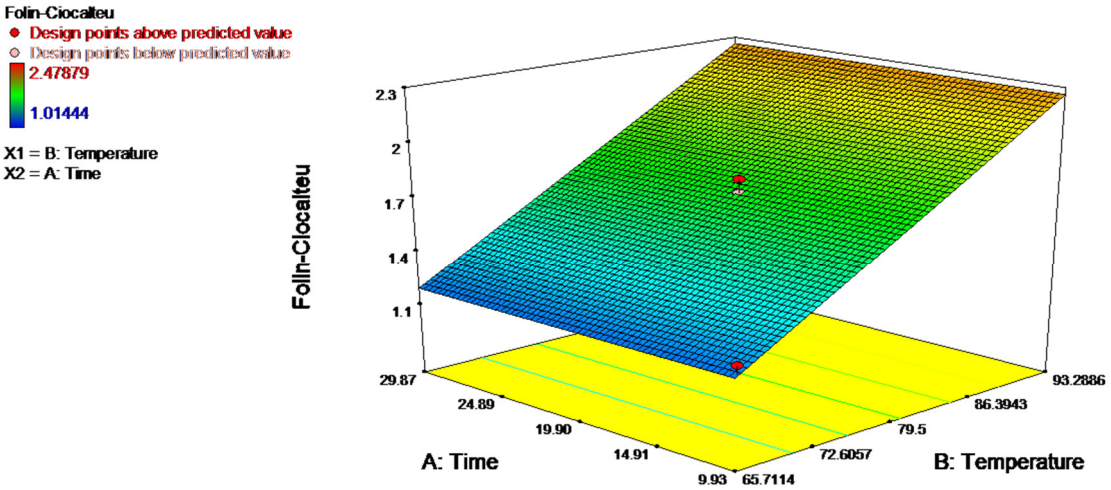


Figure S2. Model graph for fresh nettle obtained for the Folin-Ciocalteu method.

Table S3. Structures of compounds determined using capillary isotachophoresis (oxalic, citric and succinic acid) and LC-MS/MS (other compounds).

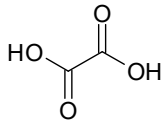
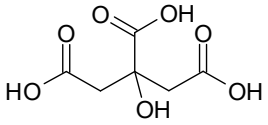
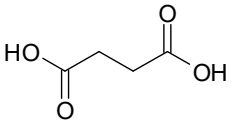
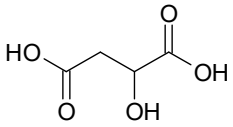
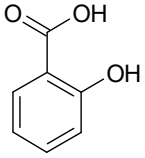
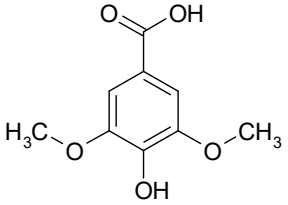
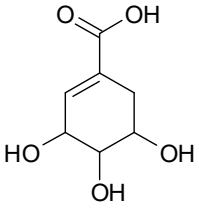
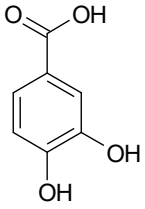
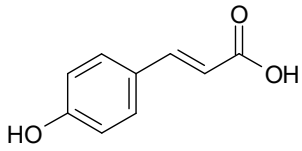
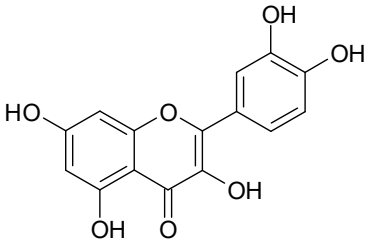
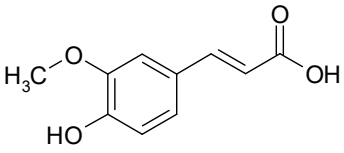
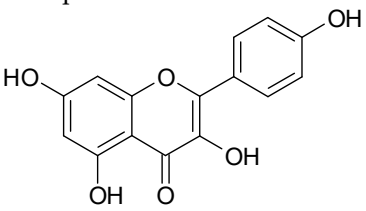
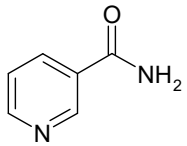
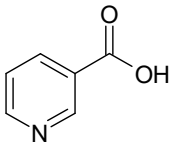
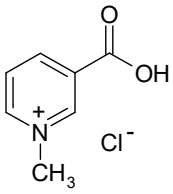
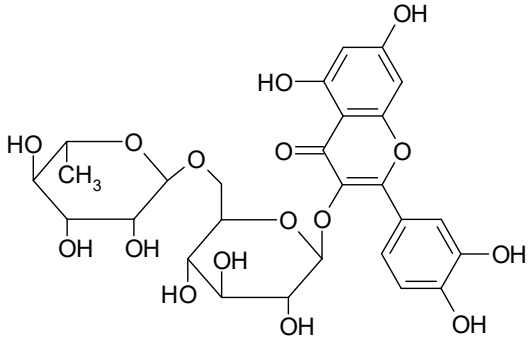
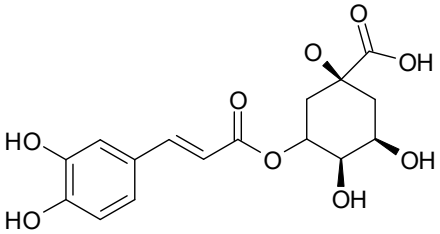
Oxalic acid 	Citric acid 	Succinic acid 
Malic acid 	Salicylic acid 	Syringic acid 
Quinic acid 	Protocatechuic acid 	*p*-coumaric acid 
Quercetin 	Ferulic acid 	Kaempferol 
Nicotinamide 	Nicotinic acid 	Trigonelline 
Rutin 	3-caffeoylquinic acid 	

Table S4. Linearity, limit of detection and limit of quantitation in the ITP method.

Analyte	Calibration Curve Range [mg L^{-1}]	Correlation Coefficient (r^2)	Limit of Detection [mg L^{-1}]	Limit of Quantitation [mg L^{-1}]
Phosphate (V)	8–500	0.9994	3	8
Oxalic acid	8–500	0.9964	3	8
Citric acid	20–500	0.9999	7	20
Malic acid	20–500	0.9996	7	20

Table S5. LC mobile phase gradient and MS source parameters.

Mobile phase Gradient (Percentage of Acetonitrile)	Parameters of Source
Negative ionization	
0 min 5%, 2 min 5%, 5 min 15%, 10 min 20% 11 min 70% 15.5 min 90%	curtain gas 20 psi nebulizer gas 45 psi auxiliary gas 50 psi temperature 450 °C collision gas medium ion spray voltage -4500 V entrance potential -10 V
Positive ionization	
0 min 10%, 2 min 10%, 2.5 min 100%, 4 min 100%	curtain gas 20 psi nebulizer gas 45 psi auxiliary gas 45 psi temperature 450 °C collision gas medium ion spray voltage 4500 V entrance potential 10 V

Table S6. Retention times and mass spectrometer parameters applied for the determination of compounds.

Compound	Retention Time [min]	DP[a] [V]	Analytical Transition	CE[b] [V]	CXP[c] [V]	Confirmatory Transition	CE [V]	CXP [V]
Negative ionization								
Quinic acid	1.11	−55	191 → 85	−31	−5	191 → 93	−31	−6
Succinic acid	1.53	−31	117 → 73	−17	−4	117 → 99	−16	−7
Gallic acid	1.95	−45	169 → 125	−22	−7	169 → 95	−56	−6
(−)-Gallocatechin	3.34	−80	305 → 125	−31	−6	305 → 167	−30	−2
Protocatechuic acid	3.46	−45	153 → 109	−22	−5	153 → 91	−37	−3
(+)-Catechin	5.95	−80	289 → 245	−23	−4	289 → 203	−29	−3
Chlorogenic acid	6.12	−40	353 → 191	−30	−2	353 → 85	−62	−5
Caffeic acid	6.47	−45	179 → 135	−25	−5	179 → 89	−47	−6
Syringic acid	6.79	−40	197 → 182	−20	−2	197 → 123	−34	−9
(−)-Epicatechin	6.97	−80	289 → 245	−23	−4	289 → 203	−29	−3
(−)-Epigallocatechin 3-gallate	7.28	−80	457 → 169	−24	−2	457 → 305	−28	−7
(−)-Gallocatechin 3-gallate	7.80	−80	457 → 169	−24	−2	457 → 305	−28	−7
p-Coumaric acid	8.07	−35	163 → 119	−26	−5	163 → 93	−47	−6
Ferulic acid	8.96	−40	193 → 134	−23	−5	193 → 178	−19	−6
Sinapic acid	9.15	−40	223 → 208	−20	−3	223 → 164	−22	−2
(−)-Epicatechin-3-gallate	9.42	−80	441 → 169	−29	−2	441 → 289	−27	−5
Rutin	9.48	−80	609 → 300	−53	−6	609 → 271	−80	−4
(−)-Catechin 3-gallate	9.86	−80	441 → 169	−29	−2	441 → 289	−27	−5
Salicylic acid	10.92	−20	137 → 93	−26	−20	137 → 65	−40	−4
Quercetin	12.38	−80	301 → 151	−31	−7	301 → 179	−26	−2
Kaempferol	12.53	−80	285 → 117	−60	−4	285 → 93	−50	−6
Positive ionization								
Trigonelline	1.04	50	138 → 94	30	4	138 → 92	30	4
Nicotinamide	1.08	15	123 → 80	28	2	123 → 96	28	4
						123 → 78	35	4
Nicotinic acid	1.12	30	124 → 80	30	3	124 → 96	29	4
						124 → 78	32	3

[a]DP – declustering potential, [b]CE – collision energy, [c]CXP – cell exit potential.

Table S7. Linearity, limit of detection and limit of quantitation in the LC-MS/MS method.

Analyte	Calibration Curve Range [µg L^{-1}]	Correlation Coefficient (r^2)	Limit of Detection [µg L^{-1}]	Limit of Quantitation [µg L^{-1}]
Quinic acid	2–4000	0.9998	0.76	2.54
Succinic acid	2–1000	0.9986	0.85	2.84
Gallic acid	2–1000	0.9992	0.40	1.32
(-)-Gallocatechin	20–4000	0.9988	0.89	2.96
Protocatechuic acid	2–1000	0.9984	0.12	0.40
(+)-Catechin	2–4000	0.9985	0.43	1.45
3-chlorogenic acid	2–1000	0.9986	0.23	0.76
Syringic acid	2–4000	0.9998	0.15	0.50
(-)-Epicatechin	2–1000	0.9996	0.24	0.78
(-)-Epigallocatechin 3-gallate	20–4000	0.9995	3.44	11.47
(-)-Gallocatechin 3-gallate	10–4000	0.9999	2.03	6.75
Ferulic acid	2–4000	0.9981	0.11	0.35
Sinapic acid	2–1000	0.9987	0.06	0.19
(-)-Epicatechin-3-gallate	2–4000	0.9996	0.73	2.45
(-)-Catechin 3-gallate	2–4000	0.9993	0.82	2.74
Salicylic acid	2–4000	0.9991	0.46	1.53
Quercetin	2–1000	0.9975	0.14	0.48
Caffeic acid	2–100	0.9996	0.51	1.71
Rutin	4–2000	0.9998	0.12	0.40
Kaempferol	4–400	0.9995	0.50	1.67
p-coumaric acid	0.5–250	0.9982	0.06	0.21
Nicotinamide	0.5–100	1.000	0.1	0.3
Nicotinic acid	1–1000	0.999	0.4	1.2
Trigonelline	0.5–50	0.998	0.04	0.1