Optimized Enzymatic Extraction of Phenolic Compounds from *Verbascum nigrum* L.: A Sustainable Approach for Enhanced Extraction of Bioactive Compounds

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Supplementary Materials

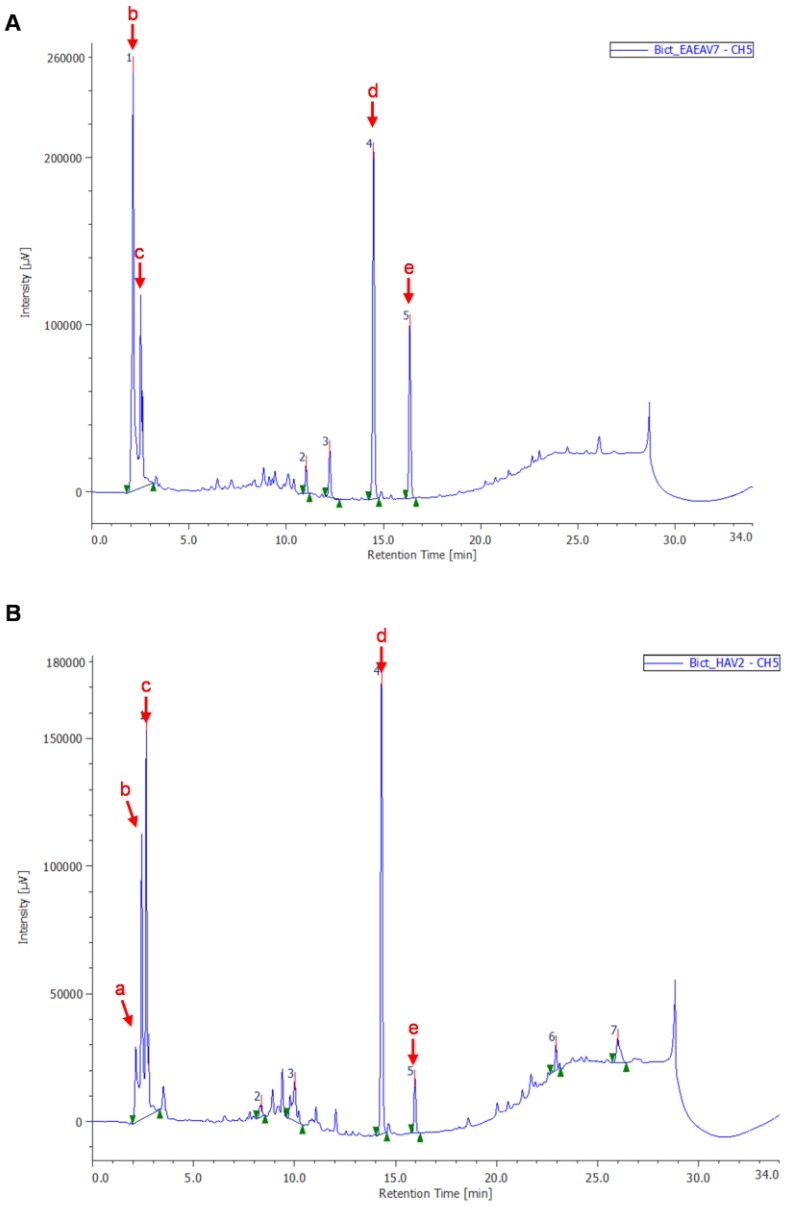


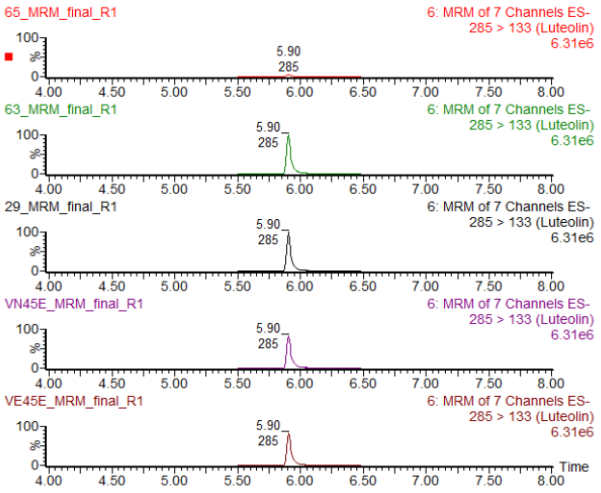
Figure S1. Preliminary analysis of the *V. nigrum* extracts obtained by enzymatic treatment followed by hydroalcoholic extraction (EH+HA) or hydroalcoholic extraction alone (HA). Two different enzymatic concentrations were assayed (15% and 30% by weight of dry biomass). HPLC chromatograms (λ = 254 nm) of the whole extract with (**A**) or without (**B**) enzymatic treatment. Peaks marked a, b, c, d, and e are attributed to phenolic species.

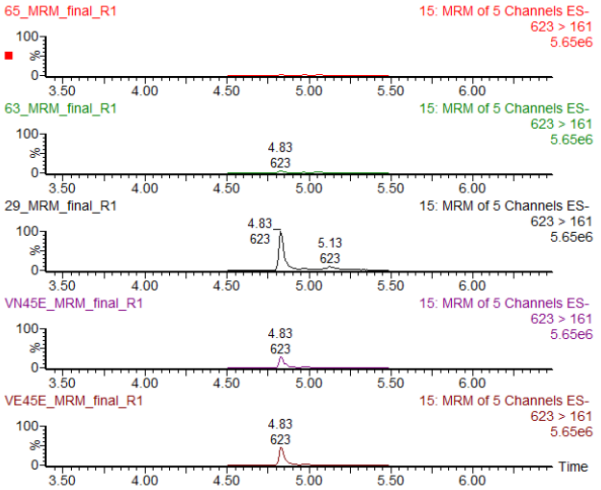
Table S1. Extraction yields of phenolic compounds and flavonoids obtained, expressed as TPC and TFC.

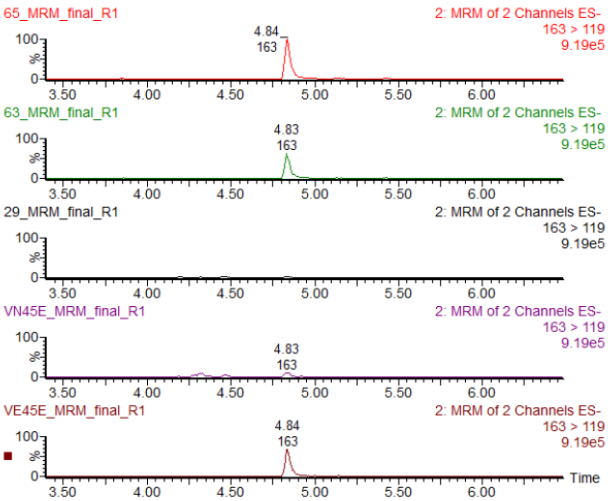
Exp No	TPC (mg/g DM)	TFC (mg/g DM)
1	23.81	23.98
2	24.14	23.32
3	22.87	23.27
4	21.60	23.10
5	25.19	20.54
6	25.91	23.90
7	24.59	22.75
8	23.83	23.23
9	23.37	22.45
10	25.00	25.64
11	21.73	20.85
12	23.81	23.78
13	24.30	23.33
14	20.99	21.95
15	20.61	22.41
16	22.85	24.01
17	22.89	22.62
18	25.39	23.98
19	25.84	25.34
20	24.83	21.76
21	25.30	24.41
22	22.45	22.31
23	25.83	22.56
24	22.69	23.47
25	24.46	25.03
26	26.03	22.25
27	23.03	24.91
28	25.46	25.22
29	25.19	22.99
30	23.37	22.21

Table S2. Total phenolic compound (TPC) and flavonoid (TFC) content of extracts obtained from *V. nigrum*. EH, enzymatic pretreatment; HA, hydroalcoholic extraction; C, cellulase; X, xylanase; P, pectinase; A, α-amylase.

Type of pretreatment	Enzyme concentration (U / g DM)	TPC (mg/g DM)	TFC (mg/g DM)
EH	100	10	1
HA	-	14	6
C	100	16	8
C	200	18	8
X	100	17	8
X	200	16	7
A	100	14	8
A	200	16	8
P	100	18	9
P	200	18	9
C+X	100	16	8
C+X	200	17	7
C+A	100	13	8
C+A	200	16	7
C+P	100	19	9
C+P	200	18	9
X+A	100	16	5
X+A	200	18	7
X+P	100	17	7
X+P	200	16	8
A+P	100	18	8
A+P	200	17	8
C+X+A	100	13	7
C+X+A	200	15	8
C+X+P	100	17	9
C+X+P	200	17	9
X+A+P	100	19	9
X+A+P	200	18	9
C+A+P	100	18	9
C+A+P	200	18	9
C+X+A+P	100	19	10
C+X+A+P	200	18	9


65_MRM_final_R1
6: MRM of 7 Channels ES-
285 > 133 (Luteolin)
6.31e6
5.90
285
63_MRM_final_R1
6: MRM of 7 Channels ES-
285 > 133 (Luteolin)
6.31e6
5.90
285
29_MRM_final_R1
6: MRM of 7 Channels ES-
285 > 133 (Luteolin)
6.31e6
5.90
285
VN45E_MRM_final_R1
6: MRM of 7 Channels ES-
285 > 133 (Luteolin)
6.31e6
5.90
285
VE45E_MRM_final_R1
6: MRM of 7 Channels ES-
285 > 133 (Luteolin)
6.31e6
5.90
285
Time


65_MRM_final_R1
15: MRM of 5 Channels ES-
623 > 161
5.65e6
63_MRM_final_R1
15: MRM of 5 Channels ES-
623 > 161
5.65e6
4.83
623
29_MRM_final_R1
15: MRM of 5 Channels ES-
623 > 161
5.65e6
4.83
623
5.13
623
VN45E_MRM_final_R1
15: MRM of 5 Channels ES-
623 > 161
5.65e6
4.83
623
VE45E_MRM_final_R1
15: MRM of 5 Channels ES-
623 > 161
5.65e6
4.83
623
Time


65_MRM_final_R1
2: MRM of 2 Channels ES-
163 > 119
9.19e5
4.84
163
63_MRM_final_R1
2: MRM of 2 Channels ES-
163 > 119
9.19e5
4.83
163
29_MRM_final_R1
2: MRM of 2 Channels ES-
163 > 119
9.19e5
VN45E_MRM_final_R1
2: MRM of 2 Channels ES-
163 > 119
9.19e5
4.83
163
VE45E_MRM_final_R1
2: MRM of 2 Channels ES-
163 > 119
9.19e5
4.84
163
Time

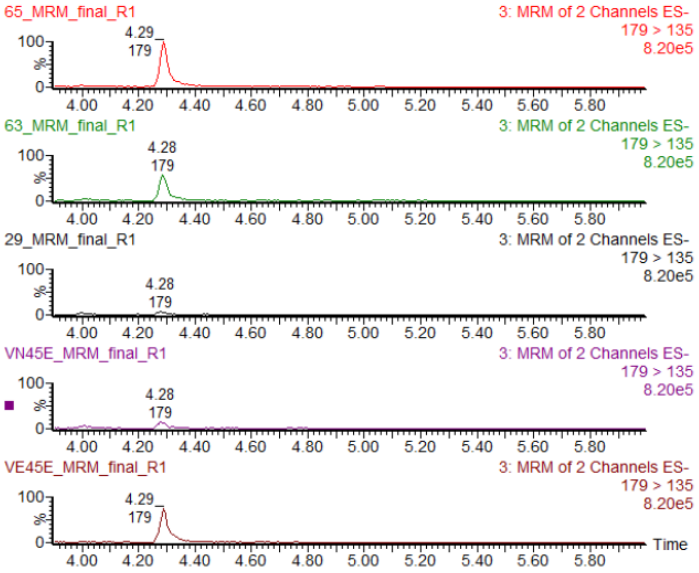


Figure S2. UPLC-ESI-MS/MS traces of the most relevant compounds: In order, luteolin; verbascoside; *p*-coumaric acid; caffeic acid.

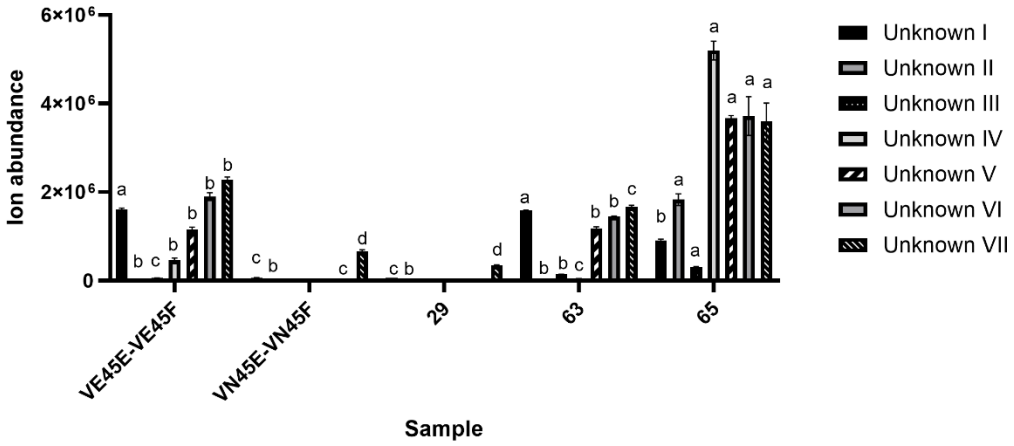


Figure S3. Ion abundance levels of unknown compounds in the *Verbascum nigrum* extracts. Ion abundance levels of components analysed through LC-MS/MS were reported as mean values ± SD. Analysis of variance (ANOVA) was carried out through Tukey's HSD test. Different letters indicate significantly different values ($p < 0.05$).