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Research Article

Supplementary Materials for the Research Article Scrutinizing the Antimicrobial and Antioxidant Potency of European Cranberry Bush (*Viburnum opulus* L.) Extracts

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Abstract: In the process of considering the documented health benefits of *Viburnum opulus* L. (*V. opulus*), including its anti-inflammatory and antioxidant activities, the present study was designed to qualitatively and quantitatively evaluate the biochemical profile and antimicrobial potency of four commercially available *V. opulus* extracts. These extracts were obtained from its flowers, bark, berries, and a mixture thereof by cold ultrasound-assisted extraction. An examination of the *V. opulus* extracts indicated a relative abundance of group compounds, such as phenolics, flavonoids, tannins, and anthocyanins, which are responsible for antioxidant activity (AOA). The widest range in all of the four group compounds was detected in the *V. opulus* extract sourced from berries, whereas the narrowest range was found in those obtained from flowers. The HPLC-ESI-TQ-MS/MS technique displayed relative fluctuations in the concentrations of individual amino acids (AAs) over the four *V. opulus* extracts. The prevalence of proline was marked in the flower-derived extract, which made up 63.3% of the total AAs, while aspartic and glutamic acids dominated in the berry-derived extract by contributing up to 29.2 and 24.4% to the total AA content, respectively. Profiling of the individual phenolic compounds disclosed the superiority of chlorogenic acid (up to 90.3%) in the berry and mixed extracts, as well as catechin (up to 57.7%) and neochlorogenic acid (11.1%) in the bark extract, which conveyed a remarkable contribution toward antimicrobial activity. The lowest content of individual phenolics was found in the flower extract. Owing to its substantially denser bioactive composition, the *V. opulus* berries and bark extracts exhibited markedly better AOA, which was pinpointed by three independent methods, i.e., DPPH$^{\bullet}$, FRAP, and ABTS$^{\bullet+}$, than those obtained from flowers or a mixture of *V. opulus* morphological parts. As part of the antimicrobial activity testing, the *V. opulus* extracts exhibited outstanding inhibitory activity and a homeopathic mode of action. The *V. opulus* extracts obtained from a mixture, bark, and berries were more active against 8 out of 19 selected test microorganisms at minimum inhibitory concentration (MIC) values that ranged from 0.24 to 0.49 μL mL^{-1}. Overall, the extracts of *V. opulus* were found to be effective against Gram-positive and Gram-negative bacteria. However, their conceivable exploitation as functional or pharmaceutical ingredients must be further clarified within in vivo models.

Keywords: antibacterial activity; catechins; chlorogenic acid; cramp bark bioactives; minimum inhibitory concentration; resistance

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Table S1. Calibration data and linearity for group compounds and antioxidant activity tests.

Compound	Linearity range, μg mL^{-1}	Calibration curve means (intercept (a) and slope (b))	Coefficient of determination (R^2)
TPC	10–100	$y = 6.8 \times 10^{-3} - 4.4 \times 10^{-3}$	0.9994
TFC	50–300	$y = 3.5 \times 10^{-3} - 9.5 \times 10^{-3}$	0.9993
TTC	5–100	$y = 1.52 \times 10^{-2} - 7.8 \times 10^{-3}$	0.9994
$DPPH^{\bullet}$	10–200	$y = 5.78 \times 10^{-1} + 1.1584$	0.9991
FRAP	10–100	$Y = 8.8 \times 10^{-3} + 7.9 \times 10^{-3}$	0.9982
$ABTS^{\bullet+}$	25–200	$y = 2.9 \times 10^{-3} - 2.2 \times 10^{-2}$	0.9952

Note: TPC – total phenolics content; TFC – total flavonoids content; TTC – total tannins content; TAC – total anthocyanins content.

Table S2. Multiple reaction monitoring (MRM) transitions, collision energy, Q1, Q3 and dwell time for investigated amino acids.

Compound	Retention time, min	Molecular formula	Ionization mode	MRM transitions	Q1 Pre Bias, V	Collision energy, V	Q3 Pre Bias, V	Dwell time, msec
Cystine	1.959	$C_6H_{12}N_2O_4S_2$	$[M+H]^+$	240.8000→74.1000	-15.0	-28.0	-16.0	16.0
				240.8000→152.1500	-29.0	-13.0	-12.0	16.0
Aspartic acid	1.997	$C_4H_7NO_4$	$[M+H]^+$	134.1000→73.9500	-15.0	-15.0	-16.0	16.0
				134.1000→88.1000	-20.0	-12.0	-25.0	16.0
Serine	2.046	$C_3H_7NO_3$	$[M+H]^+$	106.2000→60.1000	-12.0	-11.0	-26.0	16.0
				106.2000→42.2000	-20.0	-23.0	-17.0	16.0
Threonine	2.128	$C_4H_9NO_3$	$[M+H]^+$	119.9000→74.2000	-18.0	-11.0	-16.0	16.0
				119.9000→56.2000	-19.0	-15.0	-12.0	16.0
Glycine	2.135	$C_2H_5NO_2$	$[M+H]^+$	76.2000→30.2000	-12.0	-11.0	-13.0	16.0
				76.2000→31.2000	-15.0	-31.0	-13.0	16.0
Glutamic acid	2.165	$C_5H_9NO_4$	$[M+H]^+$	148.0500→84.1500	-16.0	-17.0	-18.0	16.0
				148.0500→56.1500	-16.0	-26.0	-25.0	16.0
Alanine	2.254	$C_3H_7NO_2$	$[M+H]^+$	89.9000→44.2000	-10.0	-12.0	-19.0	16.0
				89.9000→45.1500	-10.0	-31.0	-19.0	16.0
Proline	2.262	$C_5H_9NO_2$	$[M+H]^+$	116.2500→70.2000	-13.0	-16.0	-15.0	16.0
				116.2500→43.2000	-13.0	-31.0	-18.0	16.0
Histidine	2.870	$C_6H_9N_3O_2$	$[M+H]^+$	156.2500→110.1000	-10.0	-15.0	-23.0	16.0
				156.2500→83.2000	-10.0	-24.0	-18.0	16.0
Lysine	3.027	$C_6H_{14}N_2O_2$	$[M+H]^+$	146.9000→84.2000	-17.0	-18.0	-18.0	16.0
				146.9000→130.1000	-17.0	-14.0	-27.0	16.0
Arginine	3.183	$C_6H_{14}N_4O_2$	$[M+H]^+$	175.1000→70.2000	-19.0	-22.0	-14.0	16.0
				175.1000→116.1000	-20.0	-15.0	-26.0	16.0
Valine	3.206	$C_5H_{11}NO_2$	$[M+H]^+$	118.1000→72.1500	-12.0	-13.0	-15.0	39.0
				118.1000→55.0500	-13.0	-23.0	-24.0	39.0
Methionine	3.492	$C_5H_{11}NO_2S$	$[M+H]^+$	150.1000→56.0000	-16.0	-17.0	-23.0	50.0
				150.1000→104.1000	-16.0	-14.0	-12.0	50.0
Tyrosine	4.576	$C_9H_{11}NO_3$	$[M+H]^+$	182.1000→91.1500	-20.0	-28.0	-19.0	75.0
				182.1000→136.1000	-11.0	-15.0	-15.0	75.0

Contin.

Compound	Retention time, min	Molecular formula	Ionization mode	MRM transitions	Q1 Pre Bias, V	Collision energy, V	Q3 Pre Bias, V	Dwell time, msec
Leucine	5.521	$C_6H_{13}NO_2$	$[M+H]^+$	132.1000→86.1500	-14.0	-12.0	-19.0	68.0
				132.1000→44.1000	-14.0	-23.0	-18.0	68.0
Isoleucine	5.523	$C_6H_{13}NO_2$	$[M+H]^+$	132.1000→86.1500	-14.0	-11.0	-19.0	68.0
				132.1000→69.2500	-21.0	-18.0	-15.0	68.0
Phenylalanine	8.827	$C_9H_{11}NO_2$	$[M+H]^+$	165.9000→120.1000	-21.0	-14.0	-14.0	104.0
				165.9000→103.1000	-23.0	-26.0	-22.0	104.0

Table S3. Multiple reaction monitoring (MRM) transitions, collision energy, Q1, Q3 and dwell time for investigated phenolic compounds.

Compound	Retention time, min	Molecular formula	Ionization mode	MRM transitions	Q1 Pre Bias, V	Collision energy, V	Q3 Pre Bias, V	Dwell time, msec
Gallic acid	8.430	$C_7H_6O_5$	$[M-H]^-$	169.0000→124.9000	12.0	17.0	10.0	97.0
				169.0000→78.9500	12.0	24.0	15.0	97.0
Neochlorogenic acid	9.736	$C_{16}H_{18}O_9$	$[M-H]^-$	353.1000→191.0500	13.0	22.0	20.0	63.0
				353.1000→135.0000	13.0	31.0	12.0	63.0
Protocatechuic acid	11.074	$C_7H_6O_4$	$[M-H]^-$	153.2000→108.9500	10.0	16.0	20.0	21.0
				153.2000→107.9500	10.0	24.0	22.0	21.0
Chlorogenic acid	11.828	$C_{16}H_{18}O_9$	$[M-H]^-$	353.1000→191.1000	19.0	22.0	20.0	18.0
				353.1000→85.0500	13.0	43.0	16.0	18.0
(+)-Catechin	12.180	$C_{15}H_{14}O_6$	$[M-H]^-$	288.9500→245.0000	14.0	15.0	14.0	14.0
				288.9500→109.0000	14.0	26.0	19.0	14.0
(−)-Epicatechin	12.654	$C_{15}H_{14}O_6$	$[M-H]^-$	289.0500→245.0000	14.0	16.0	14.0	14.0
				289.0500→109.0000	14.0	26.0	20.0	14.0
Vanillin	12.974	$C_8H_8O_3$	$[M+H]^+$	152.9500→65.1000	-10.0	-24.0	-24.0	14.0
				152.9500→93.0500	-10.0	-16.0	-20.0	14.0
Caffeic Acid	13.442	$C_9H_8O_4$	$[M-H]^-$	179.1500→135.0000	12.0	18.0	25.0	23.0
				179.1500→134.0000	12.0	25.0	24.0	23.0
Rutin	14.470	$C_{27}H_{30}O_{16}$	$[M-H]^-$	609.1000→300.0000	20.0	28.0	17.0	23.0
				609.1000→301.0500	21.0	36.0	17.0	23.0
para-Coumaric acid	14.579	$C_9H_8O_3$	$[M-H]^-$	163.0500→119.0500	11.0	16.0	21.0	23.0
				163.0500→93.0500	12.0	31.0	17.0	23.0
Luteolin-7-*O*-glucoside	14.717	$C_{21}H_{20}O_{11}$	$[M-H]^-$	447.0500→285.0500	20.0	28.0	17.0	23.0
				447.0500→284.0000	21.0	36.0	17.0	23.0
Vanillic acid	15.187	$C_8H_8O_4$	$[M-H]^-$	167.1500→151.9500	18.0	17.0	27.0	23.0
				167.1500→107.8000	11.0	17.0	17.0	23.0
Sinapic acid	17.448	$C_{11}H_{12}O_5$	$[M-H]^-$	223.3000→208.0000	17.0	14.0	12.0	23.0
				223.3000→192.9500	10.0	22.0	19.0	23.0
trans-Ferulic Acid	19.884	$C_{10}H_{10}O_4$	$[M-H]^-$	193.0500→134.0000	10.0	18.0	23.0	23.0
				193.0500→178.0500	10.0	15.0	15.0	23.0
Isorhamnetin	21.570	$C_{16}H_{12}O_7$	$[M+H]^+$	316.8500→252.8500	-20.0	-14.0	-19.0	70.0
				316.8500→302.1000	-20.0	-25.0	-13.0	70.0
Kaempferol	21.831	$C_{15}H_{10}O_6$	$[M+H]^+$	286.8500→222.9500	-18.0	-13.0	-17.0	70.0
				286.8500→69.1000	-18.0	-45.0	-14.0	70.0
Quercetin	20.644	$C_{15}H_{10}O_7$	$[M+H]^+$	302.8000→153.0500	-19.0	-34.0	-18.0	46.0
				302.8000→229.1000	-14.0	-30.0	-18.0	46.0
Rhamnetin	22.553	$C_{16}H_{12}O_7$	$[M-H]^-$	315.0500→170.7500	15.0	12.0	15.0	70.0
				315.0500→300.0500	22.0	22.0	19.0	70.0

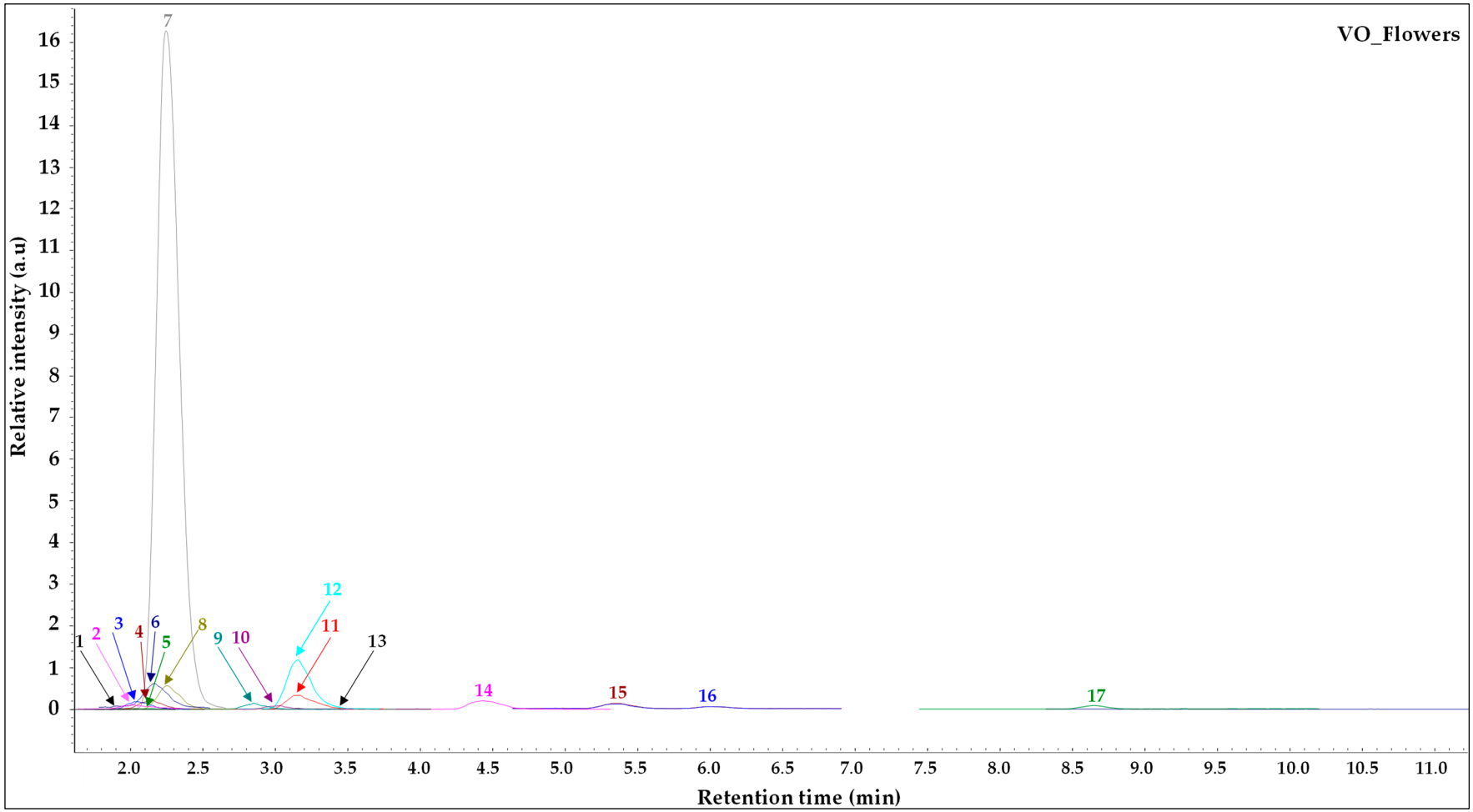

Figure S1. Extracted ion chromatogram (EIC) in multiple reaction monitoring (MRM) represents the profile of 17 multiple amino acids identified in the extract derived from flowers of *Viburnum opulus* L. ***Note*:** 1 – Cystine; 2 – Aspartic acid; 3 – Serine; 4 – Threonine; 5 – Glycine; 6 – Glutamic acid; 7 – Proline; 8 – Alanine; 9 – Histidine; 10 – Lysine; 11 – Valine; 12 – Arginine; 13 – Methionine; 14 – Tyrosine; 15 – Isoleucine; 16 – Leucine; 17 – Phenylalanine.

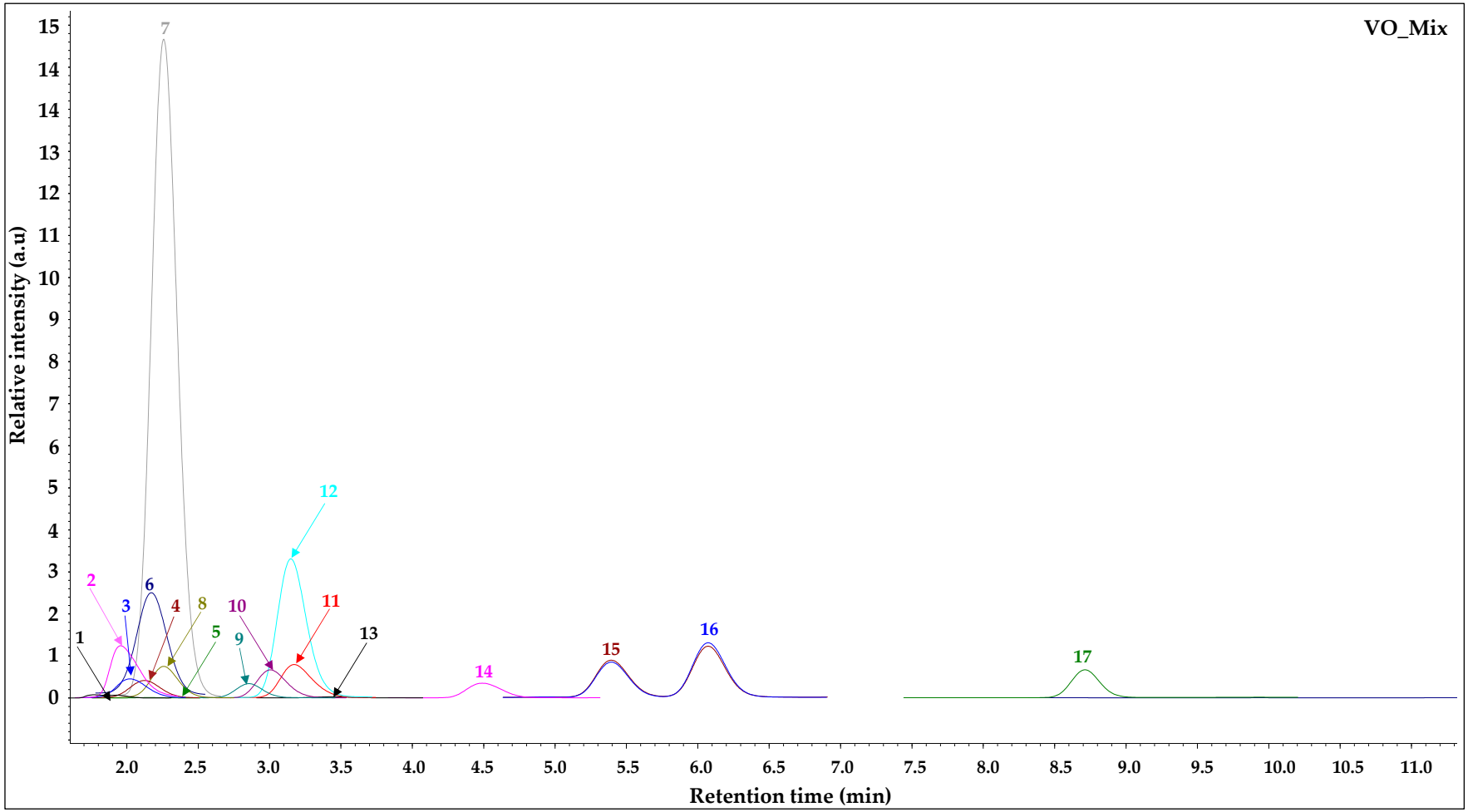

Figure S2. Extracted ion chromatogram (EIC) in multiple reaction monitoring (MRM) represents the profile of 17 multiple amino acids identified in the extract derived from mixture of morphological parts (berries without seeds, leaves, buds and bark) of *Viburnum opulus* L. ***Note*:** 1 – Cystine; 2 – Aspartic acid; 3 – Serine; 4 – Threonine; 5 – Glycine; 6 – Glutamic acid; 7 – Proline; 8 – Alanine; 9 – Histidine; 10 – Lysine; 11 – Valine; 12 – Arginine; 13 – Methionine; 14 – Tyrosine; 15 – Isoleucine; 16 – Leucine; 17 – Phenylalanine.

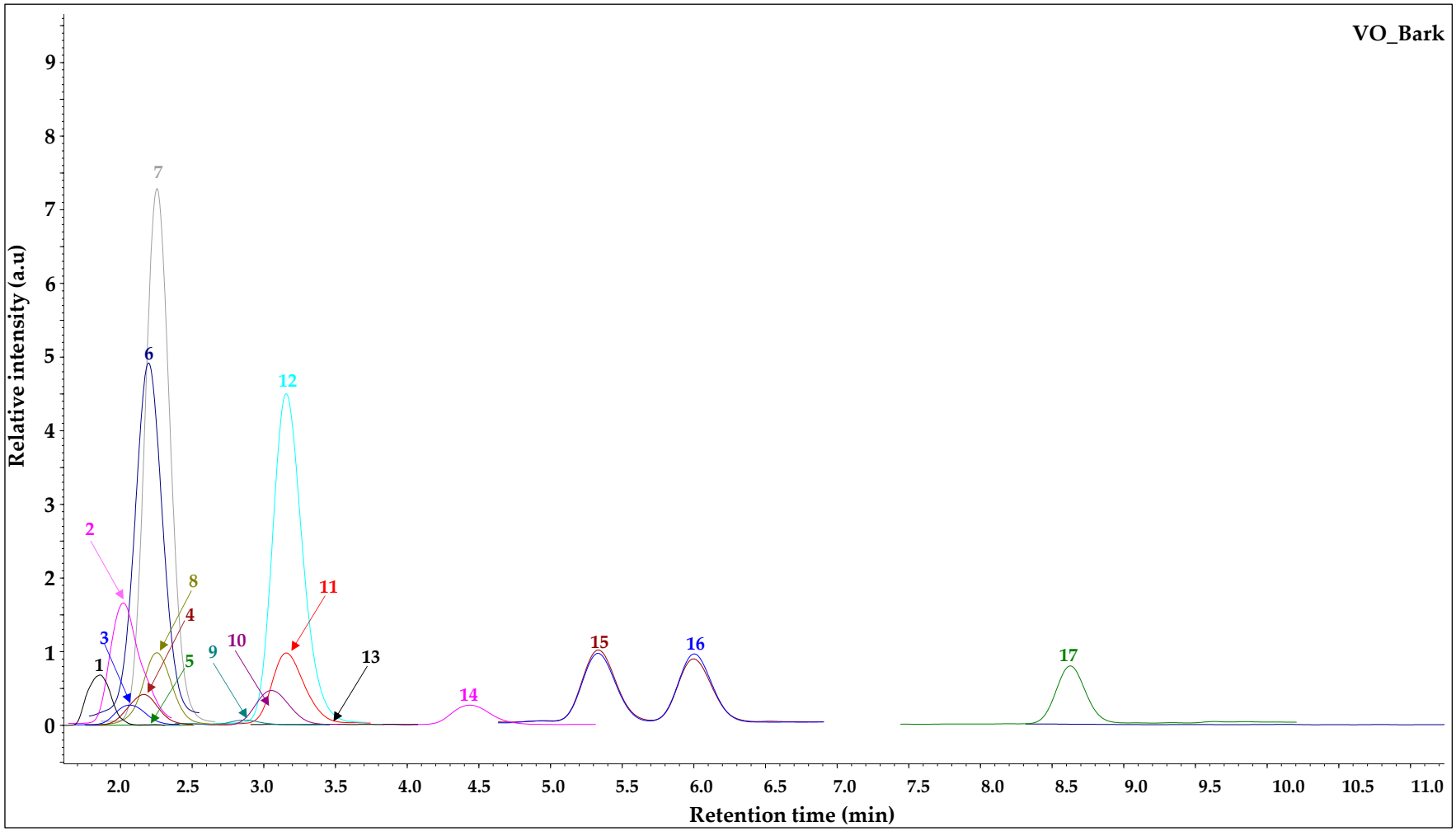

Figure S3. Extracted ion chromatogram (EIC) in multiple reaction monitoring (MRM) represents the profile of 17 multiple amino acids identified in the extract derived from bark of *Viburnum opulus* L. ***Note*:** 1 – Cystine; 2 – Aspartic acid; 3 – Serine; 4 – Threonine; 5 – Glycine; 6 – Glutamic acid; 7 – Proline; 8 – Alanine; 9 – Histidine; 10 – Lysine; 11 – Valine; 12 – Arginine; 13 – Methionine; 14 – Tyrosine; 15 – Isoleucine; 16 – Leucine; 17 – Phenylalanine.

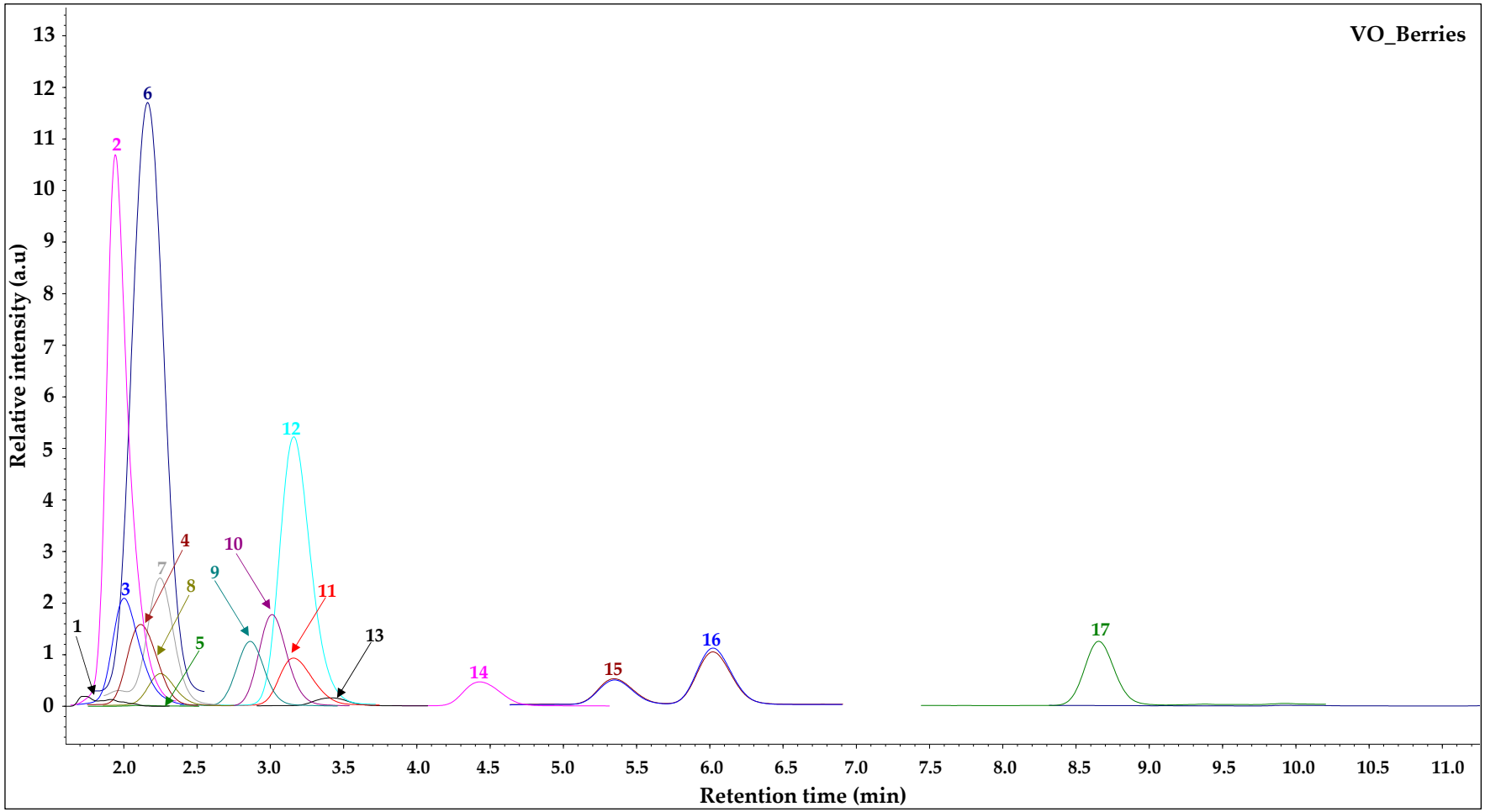

Figure S4. Extracted ion chromatogram (EIC) in multiple reaction monitoring (MRM) represents the profile of 17 multiple amino acids identified in the extract derived from berries of *Viburnum opulus* L. ***Note*:** 1 – Cystine; 2 – Aspartic acid; 3 – Serine; 4 – Threonine; 5 – Glycine; 6 – Glutamic acid; 7 – Proline; 8 – Alanine; 9 – Histidine; 10 – Lysine; 11 – Valine; 12 – Arginine; 13 – Methionine; 14 – Tyrosine; 15 – Isoleucine; 16 – Leucine; 17 – Phenylalanine.

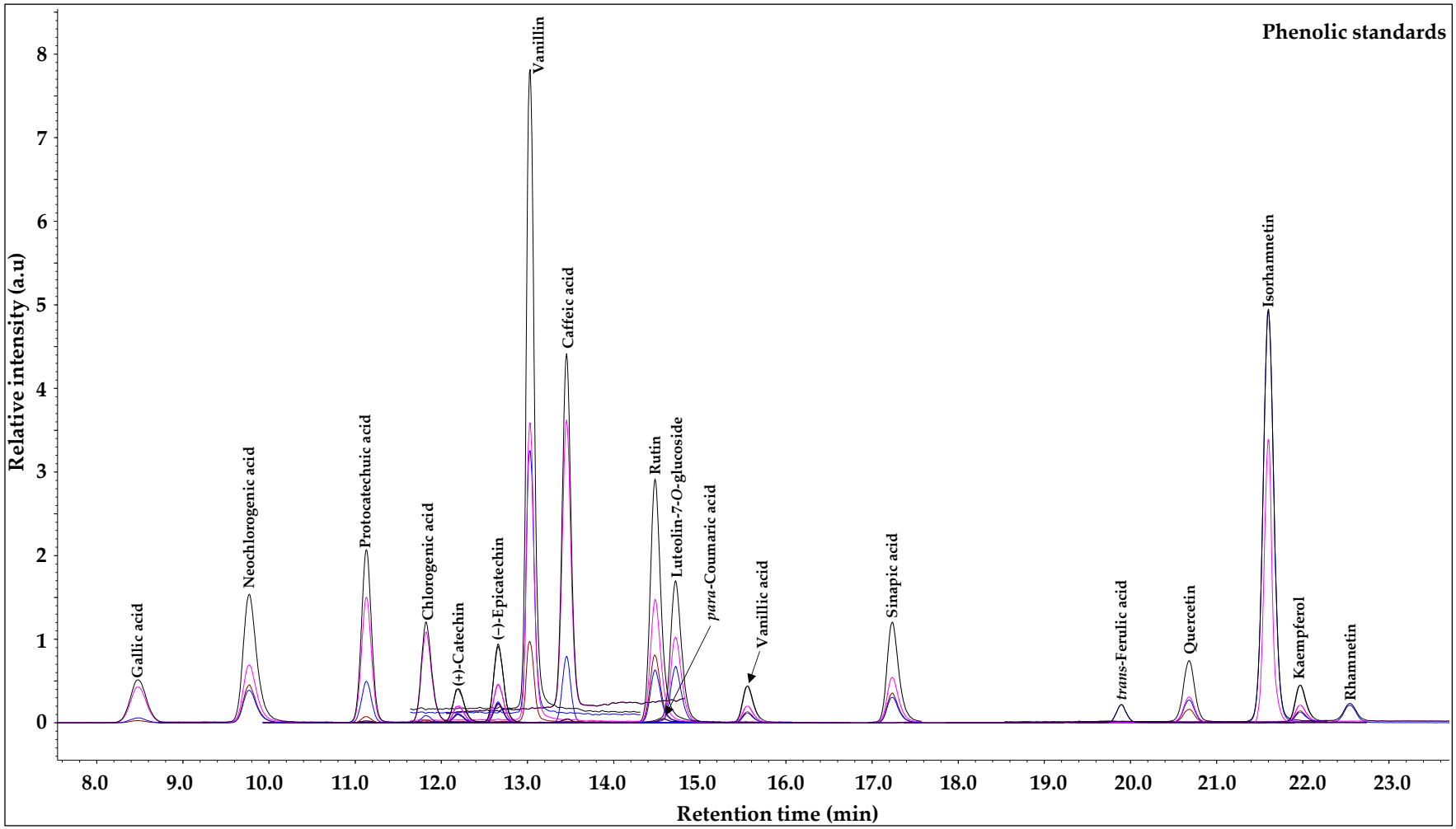

Figure S5. Extracted ion chromatogram (EIC) in multiple reaction monitoring (MRM) mode represents the profile of 18 phenolic standards at the concentration of 1 μg mL^{-1}.

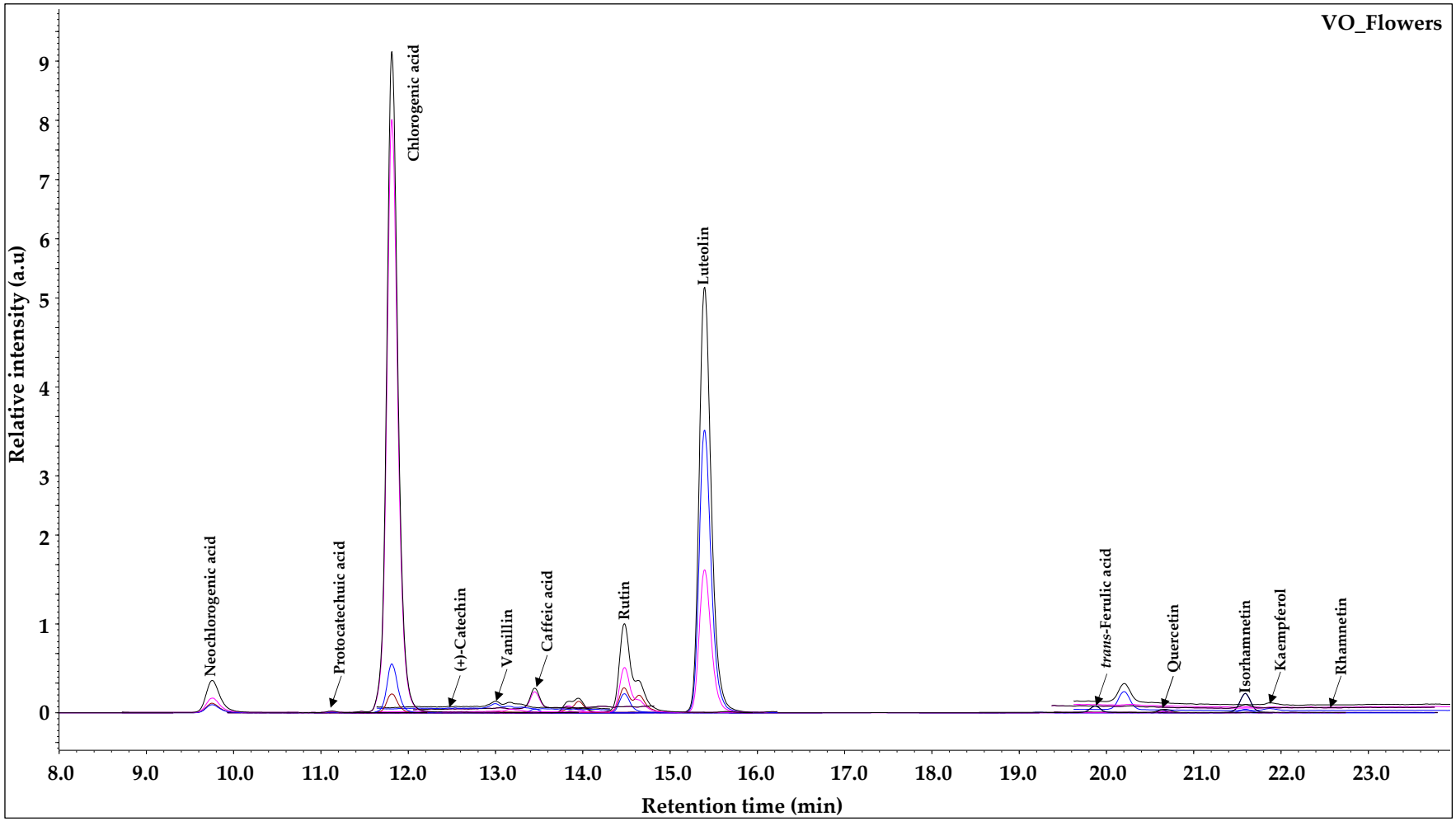

Figure S6. Extracted ion chromatogram (EIC) in multiple reaction monitoring (MRM) represents the profile of major phenolic compounds identified in the extract derived from flowers of *Viburnum opulus* L.

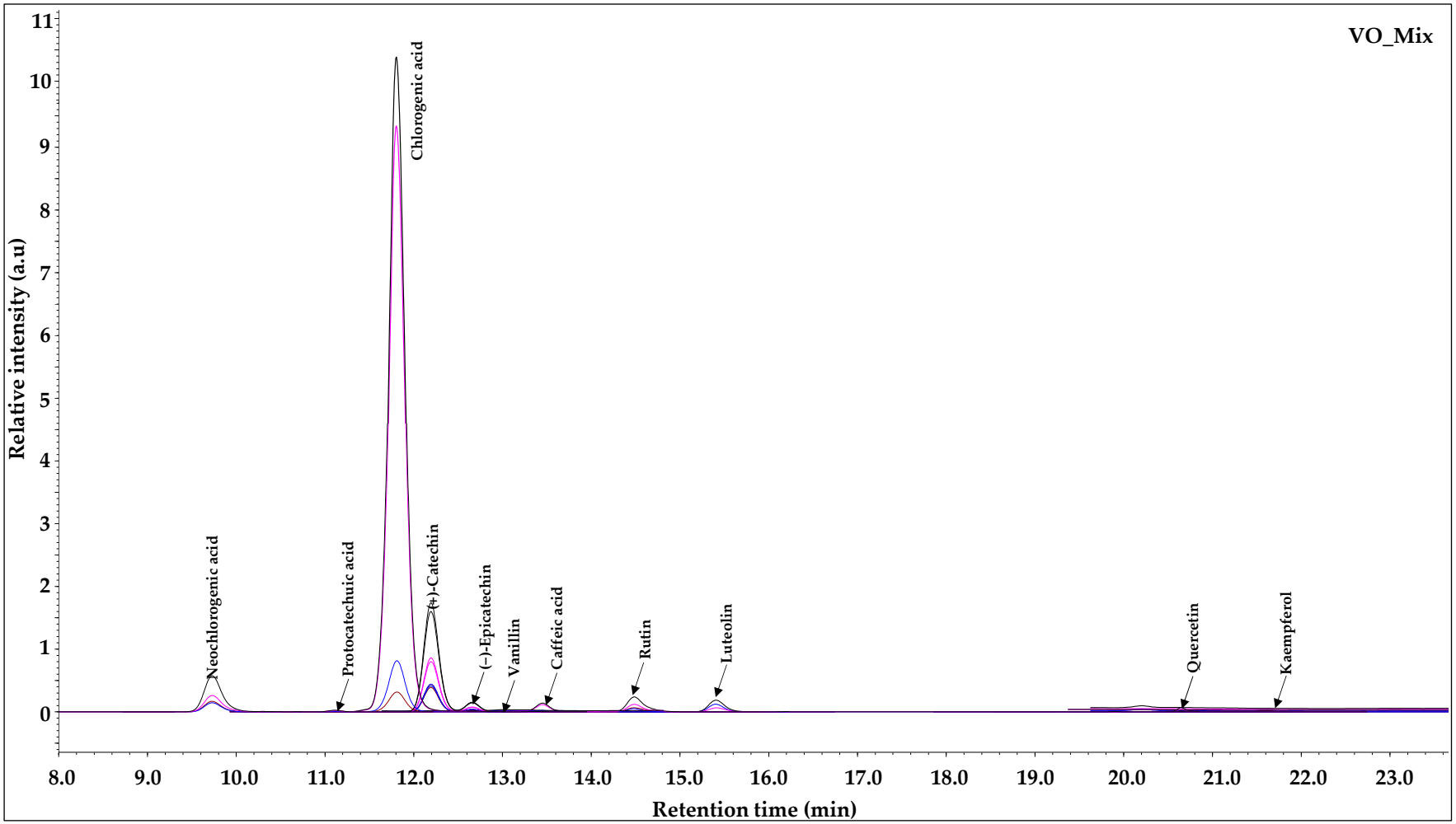

Figure S7. Extracted ion chromatogram (EIC) in multiple reaction monitoring (MRM) represents the profile of major phenolic compounds identified in the extract derived from mixture of morphological parts (berries without seeds, leaves, buds and bark) of *Viburnum opulus* L.

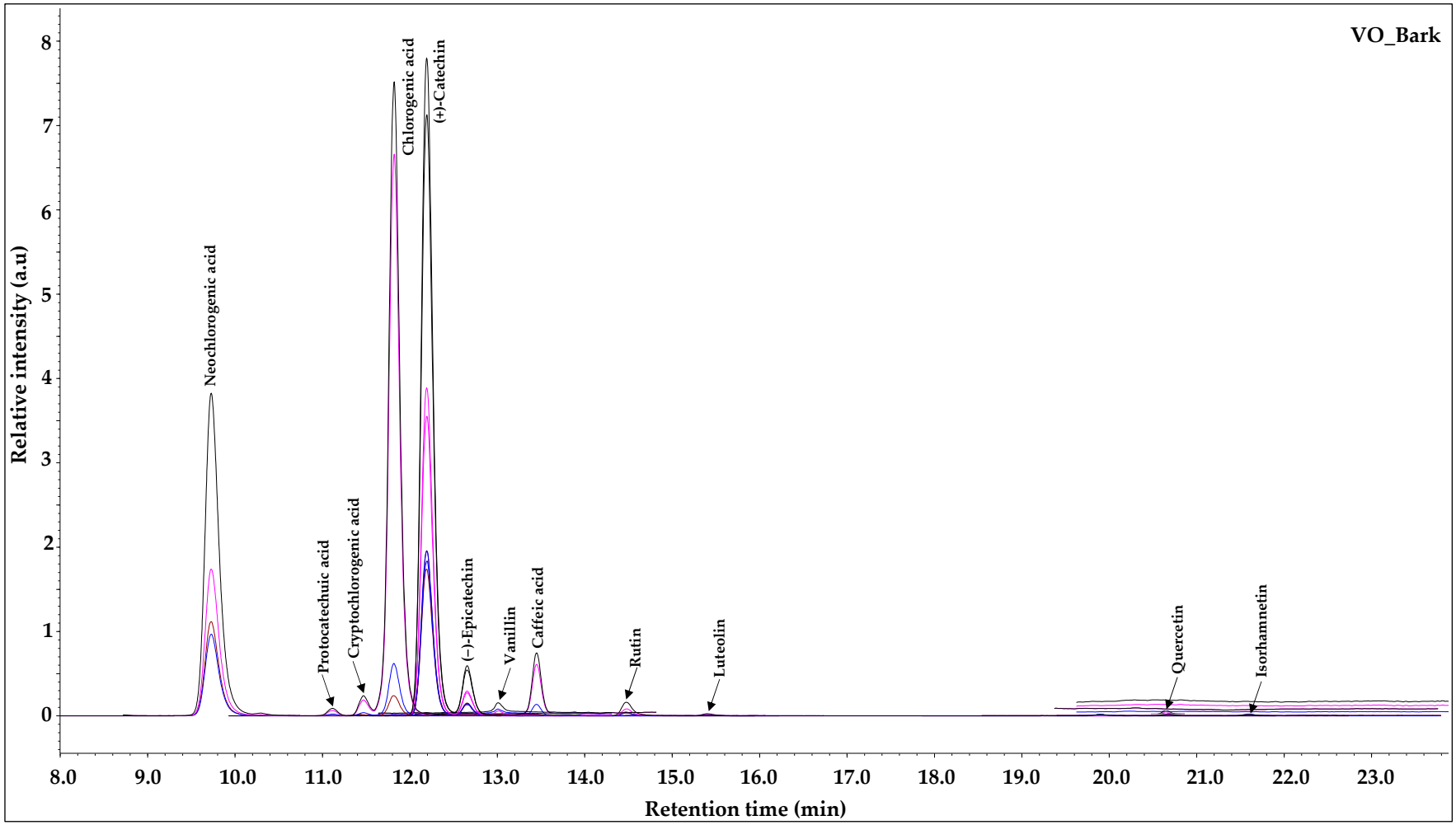

Figure S8. Extracted ion chromatogram (EIC) in multiple reaction monitoring (MRM) represents the profile of major phenolic compounds identified in the extracts derived from bark of *Viburnum opulus* L.

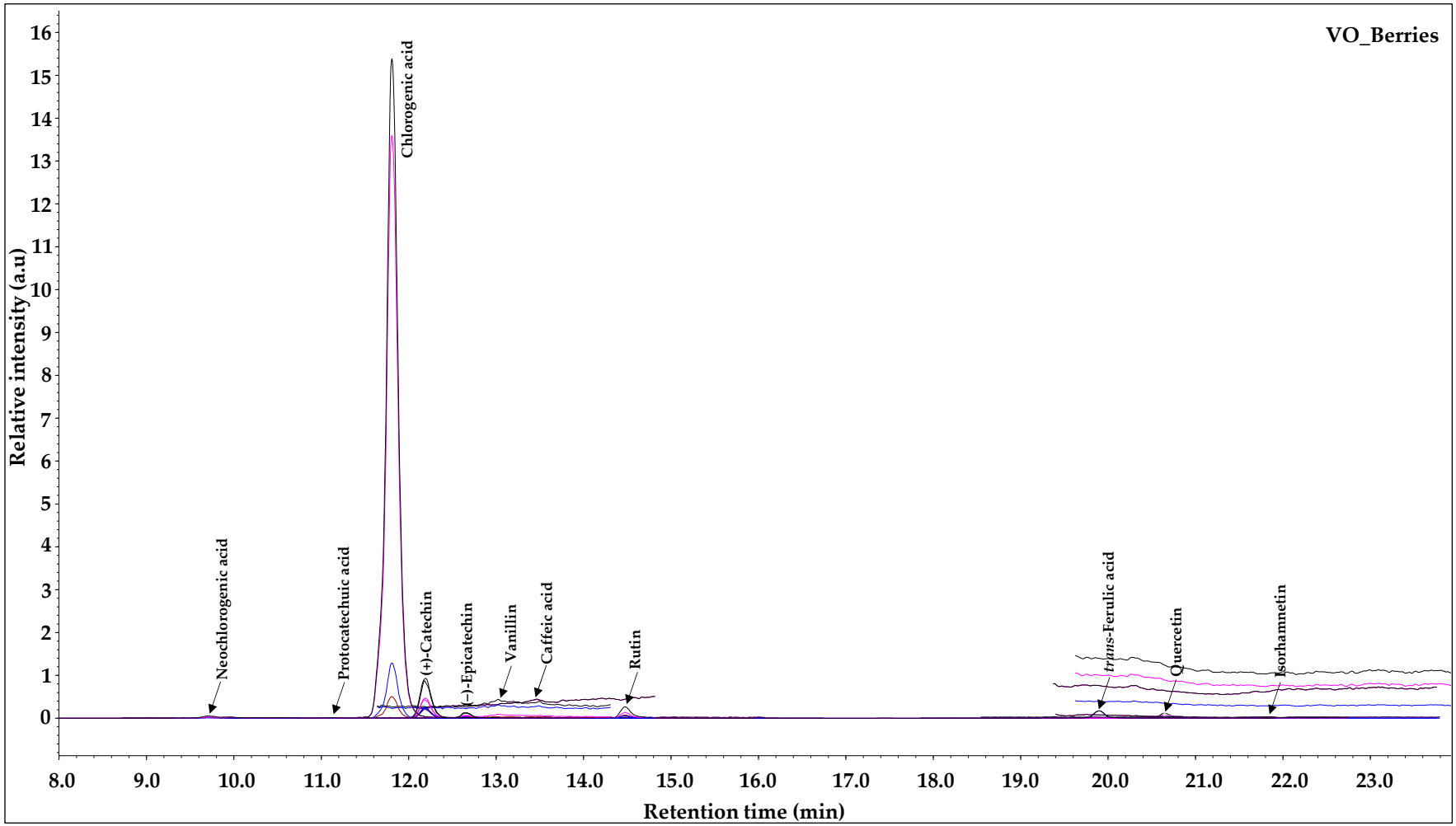

Figure S9. Extracted ion chromatogram (EIC) in multiple reaction monitoring (MRM) represents the profile of major phenolic compounds identified in the extracts derived from berries of *Viburnum opulus* L.