

Supplementary data for article:

Čukanović, J.; Tešević, V.; Jadranin, M.; Ljubojević, M.; Mladenović, E.; Kostić, S. Horse Chestnut (*Aesculus Hippocastanum* L.) Seed Fatty Acids, Flavonoids and Heavy Metals Plasticity to Different Urban Environments. *Biochemical Systematics and Ecology* **2020**, *89*.
<https://doi.org/10.1016/j.bse.2019.103980>

1 **SUPPLEMENTARY MATERIAL**

2 HORSE CHESTNUT (*Aesculus hippocastanum* L.) SEED FATTY ACIDS, FLAVONOIDS AND HEAVY
3 METALS PLASTICITY TO DIFFERENT URBAN ENVIRONMENTS

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5 **Supplementary Table legend:**

6 Supplementary Table S1. Content and ratio of saturated, unsaturated and polyunsaturated fatty acids in chestnut
7 samples (%).

8 Supplementary Table S2. Contents of phenolics ($\mu\text{g/g}$) in horse chestnut samples.

9 Supplementary Table S3. Aescin content in horse chestnut samples.

10 Supplementary Table S4. Principal Component Analysis (PCA) table of the Flavonoids and Aescin, showing
11 correlation coefficients for variables loaded on the three principal components (PCs) extracted from the analysis
12 of data from 11 genotypes.

13 Supplementary Table S5. Seven heavy metals concentration (μg) in seed content of 11 genotypes.

14 Supplementary Table S6. Principal Component Analysis (PCA) table of the seven heavy metals, showing
15 correlation coefficients for variables loaded on the three principal components (PCs) extracted from the analysis
16 of data from 11 genotypes.

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18 **Supplementary Figure legend:**

19 Supplementary Figure S1. Obtained chromatograms for the flavonoids extracts of horse chesnut seed.

20 Supplementary Figure S2. Obtained chromatograms for the flavonoids extracts of horse chesnut seed

21 Supplementary Figure S3. Obtained chromatograms for the flavonoids extracts of horse chesnut seed

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25 Supplementary Table S1. Content and ratio of saturated, unsaturated and polyunsaturated fatty acids in chestnut

26 samples (%).

Genotype	Saturated fatty acids (SAFAs)	Unsaturated fatty acids (MUFAs)	Polyunsaturated fatty acids (PUFAs)	SAFAs / MUFAs*	PUFAs / MUFAs*
BP ₂	16.60	56.40	26.40	3.39	1.59
BP ₄	17.10	62.40	20.30	3.65	1.18
BP ₂₇	16.70	52.10	30.80	3.12	1.84
BDS ₁	28.50	63.50	1.80	2.23	0.06
BDS ₂	29.20	62.60	0.70	2.14	0.02
P ₁	20.60	62.10	13.70	3.01	0.66
P ₄	20.70	65.10	11.90	3.14	0.57
P ₅	17.50	62.30	20.10	3.56	1.14
SK ₁	16.40	54.10	25.70	3.30	1.56
SK ₃	16.70	56.50	26.50	3.38	1.58
SK ₉	17.70	55.10	27.10	3.11	1.53
\bar{x} All	19.01	58.44	20.64	3.16	1.19

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29 Supplementary Table S2. Contents of phenolics ($\mu\text{g/g}$) in horse chestnut samples.

Genotype	Quercetin	Kaempferol	Rutin
BP₂	/ ^{a*}	0.155 ^{bc}	/ ^e
BP₄	0.363 ^{de}	0.129 ^{abc}	5.569 ^{cd}
BP₂₇	0.413 ^{ef}	0.119 ^{abc}	4.279 ^b
BDS₁	0.084 ^{ab}	0.060 ^{ab}	9.961 ^g
BDS₂	0.098 ^{ab}	/ ^a	1.155 ^a
P₁	0.241 ^{cd}	/ ^a	4.567 ^b
P₄	0.538 ^f	/ ^a	5.162 ^c
P₅	0.283 ^{cde}	0.429 ^d	25.784 ^h
SK₁	0.806 ^g	0.227 ^c	5.878 ^d
SK₃	0.170 ^{bc}	0.019 ^a	0.982 ^a
SK₉	/ ^a	/ ^a	3.553 ^f
\bar{x}_{All}	0.271	0.103	6.071
CV_{All} (%)	91.61	95.66	93.15

30 *Means followed by different letters differ significantly, based on Duncan's test at $p < 0.05$.31 *Legend:* /-absent

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33 Supplementary Table S3. Aescin content in horse chestnut samples.

Genotype	Seed mass (g)	Ethanol extract mass (g)	Ethanol extract in seed (%)	Aescin in ethanol extract (%)	Aescin content in seed (%)
BP ₂	11.09	2.09	18.93	21.32	4.04 ^{c*}
BP ₄	6.97	1.04	15.01	15.29	2.29 ^b
BP ₂₇	7.81	1.41	18.10	9.25	1.67 ^{ab}
BDS ₁	9.52	1.46	14.99	12.88	1.93 ^{ab}
BDS ₂	7.52	1.37	18.22	11.97	2.18 ^b
P ₁	11.70	1.51	12.94	7.16	0.93 ^a
P ₄	8.11	1.91	23.55	7.85	1.85 ^{ab}
P ₅	8.80	1.78	20.24	15.28	4.16 ^c
SK ₁	6.11	1.44	23.70	7.60	1.80 ^{ab}
SK ₃	21.68	0.78	3.60	13.14	1.26 ^a
SK ₉	5.00	0.77	15.56	5.24	0.82 ^a
\bar{x}_{All}	9.48	1.41	16.80	11.54	2.07
Cv _{All} (%)	47.52	29.97	33.15	40.77	52.03

34 *Means followed by different letters differ significantly, based on Duncan's test at $p < 0.05$.

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37 Supplementary Table S4. Principal Component Analysis (PCA) table of the Flavonoids and Aescin, showing
38 correlation coefficients for variables loaded on the three principal components (PCs) extracted from the analysis
39 of data from 11 genotypes.

Variable	PC1	PC2	PC3
Quercetin	-0.24	0.94*	-0.20
Kaempferol	-0.96*	0.07	-0.05
Rutin	-0.85*	0.03	0.51
Aescin	-0.79*	-0.42	-0.42
Eigenvalue	2.35	0.71	0.48
Var. (%)	58.74	26.92	12.09
Cum. (%)	58.74	85.66	100.00

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42 Supplementary Table S5. Seven heavy metals concentration (μg) in seed content of 11 genotypes.

Genotype	Al	Cr	Cu	Fe	Mn	Ni	Zn	Total
BP ₂	1.77 ^{abcd*}	0.05 ^a	8.60 ^{abc}	7.60 ^{ab}	1.80 ^a	0.50 ^a	9.00 ^{abcd}	29.32
BP ₄	0.59 ^{ab}	0.05 ^a	6.60 ^{cd}	6.50 ^a	2.30 ^a	0.50 ^a	7.40 ^a	23.94
BP ₂₇	0.50 ^a	0.05 ^a	10.0 ^b	7.80 ^{abc}	3.10 ^a	0.50 ^a	8.50 ^{abc}	30.45
BDS ₁	0.70 ^{ab}	0.05 ^a	7.00 ^{cde}	6.90 ^{ab}	2.20 ^a	0.50 ^a	9.80 ^{bcd}	27.15
BDS ₂	1.40 ^{abc}	0.05 ^a	5.50 ^c	6.20 ^a	2.00 ^a	0.50 ^a	8.00 ^{ab}	23.65
P ₁	1.02 ^{ab}	0.20 ^b	13.00 ^f	13.20 ^f	3.20 ^a	0.80 ^{bc}	18.10 ^f	49.52
P ₄	2.54 ^{cd}	0.10 ^a	8.00 ^{ade}	7.20 ^{ab}	2.50 ^a	0.50 ^a	7.90 ^a	28.74
P ₅	2.88 ^d	0.10 ^a	11.70 ^f	9.50 ^{cd}	2.50 ^a	0.60 ^{ab}	10.80 ^d	38.08
SK ₁	0.50 ^a	0.05 ^a	9.80 ^{ab}	8.40 ^{bc}	2.10 ^a	0.60 ^{ab}	10.0 ^{cd}	31.45
SK ₃	0.50 ^a	0.05 ^a	9.00 ^{ab}	11.30 ^e	1.80 ^a	0.60 ^{ab}	8.10 ^{ab}	31.35
SK ₉	1.9 ^{bcd}	0.10 ^a	12.4 ^f	10.40 ^{de}	3.10 ^a	0.85 ^c	13.60 ^e	42.35
\bar{x} All	1.30	0.07	9.23	8.63	2.33	0.59	10.11	32.26
CV _{All} (%)	78.04	73.01	27.00	26.65	40.2	26.73	31.55	

43 *Means followed by the same letters within a variable are not significantly different ($p < 0.05$).

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45 Supplementary Table S6. Principal Component Analysis (PCA) table of the seven heavy metals, showing
46 correlation coefficients for variables loaded on the three principal components (PCs) extracted from the analysis
47 of data from 11 genotypes.

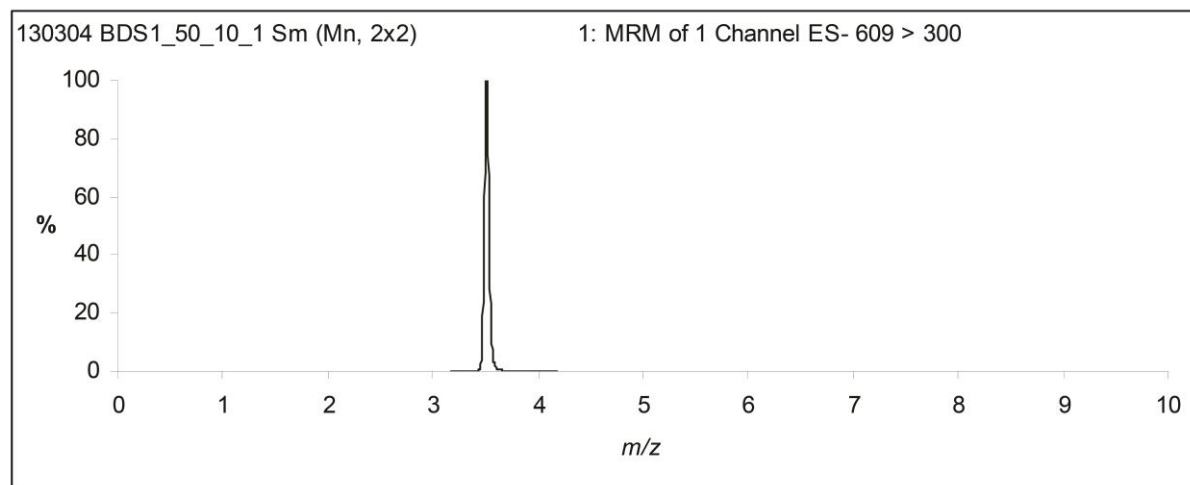
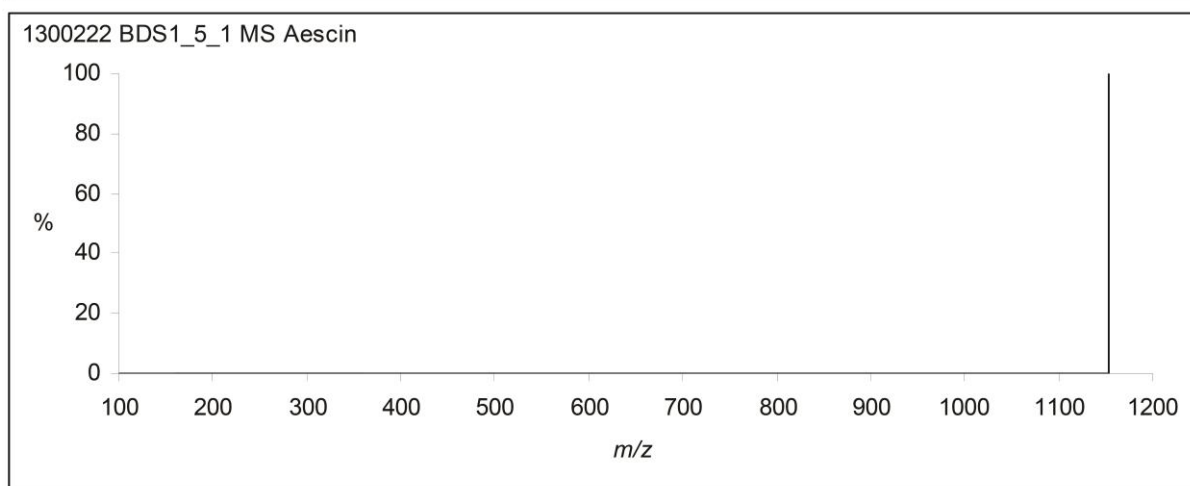
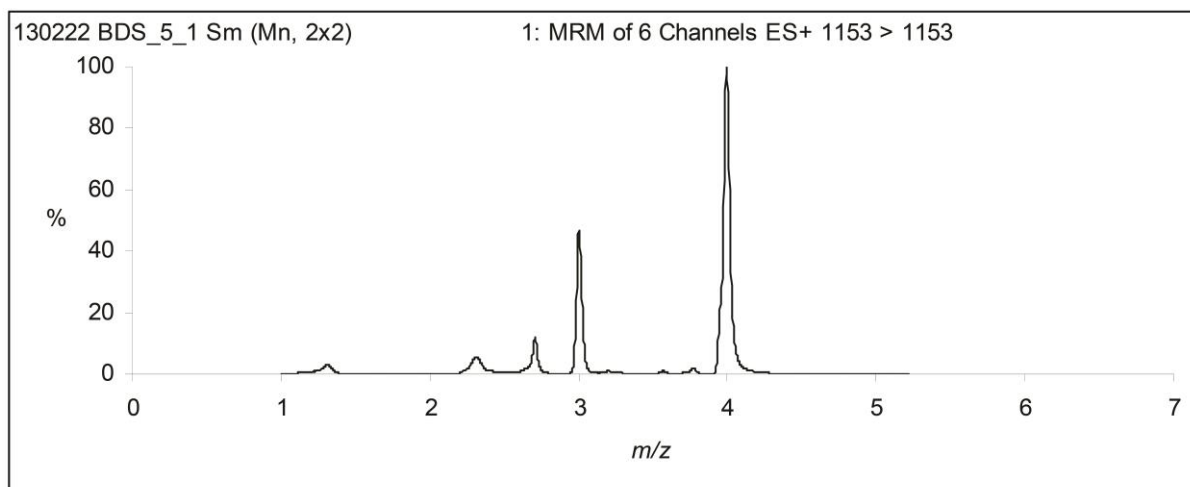
Variable	PC1	PC2	PC3
Al	-0.21	-0.96*	0.15
Cr	-0.89*	-0.18	-0.05
Cu	-0.91*	-0.02	0.06
Fe	-0.87*	0.23	0.35
Mn	-0.74*	-0.04	-0.64
Ni	-0.91*	0.12	0.14
Zn	-0.94*	0.09	/
Eigenvalue	4.68	1.04	0.59
Var. (%)	66.96	14.96	8.50
Cum. (%)	66.96	81.92	90.43

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51 Supplementary Figure S1. Obtained chromatograms for the flavonoids extracts of horse chesnut seed

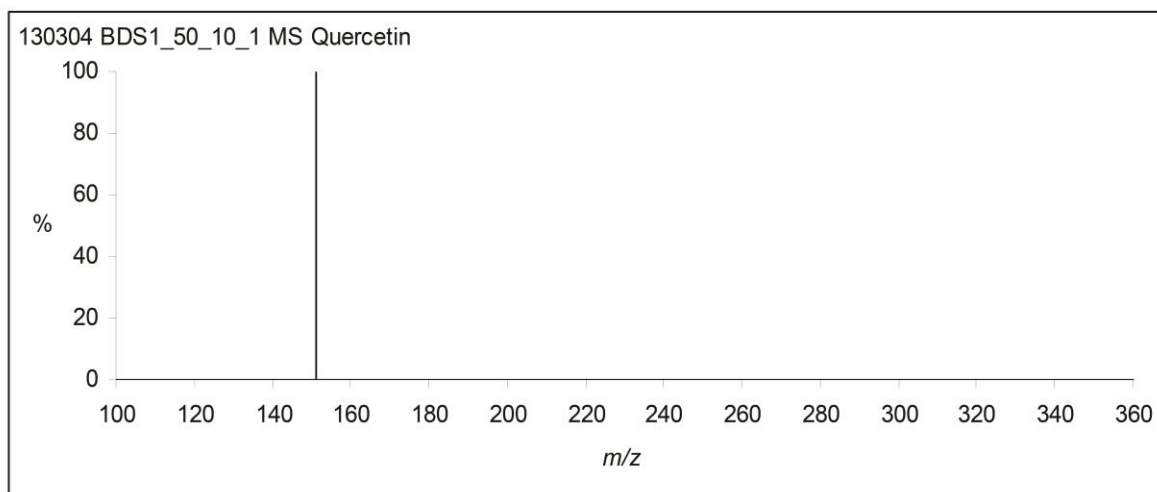
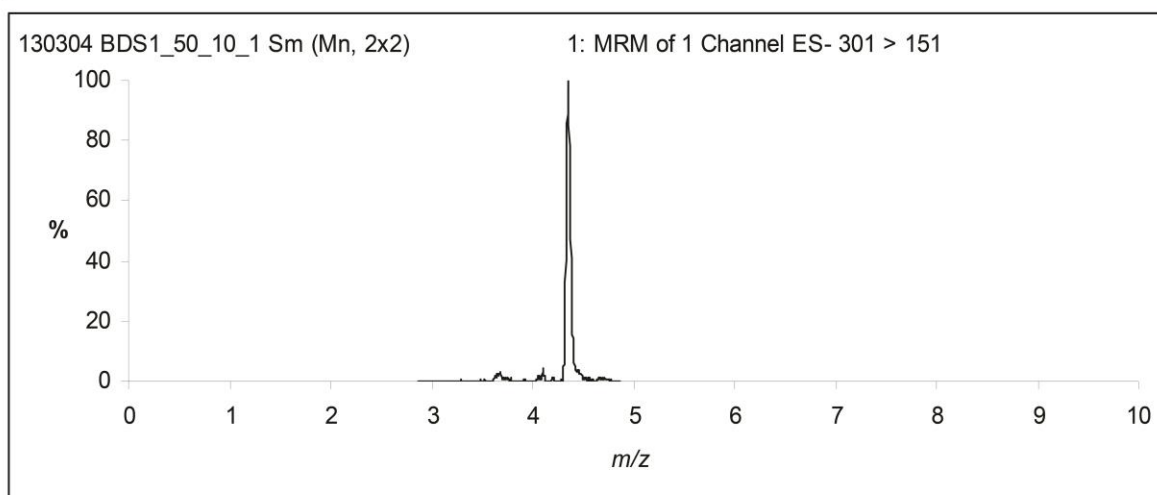
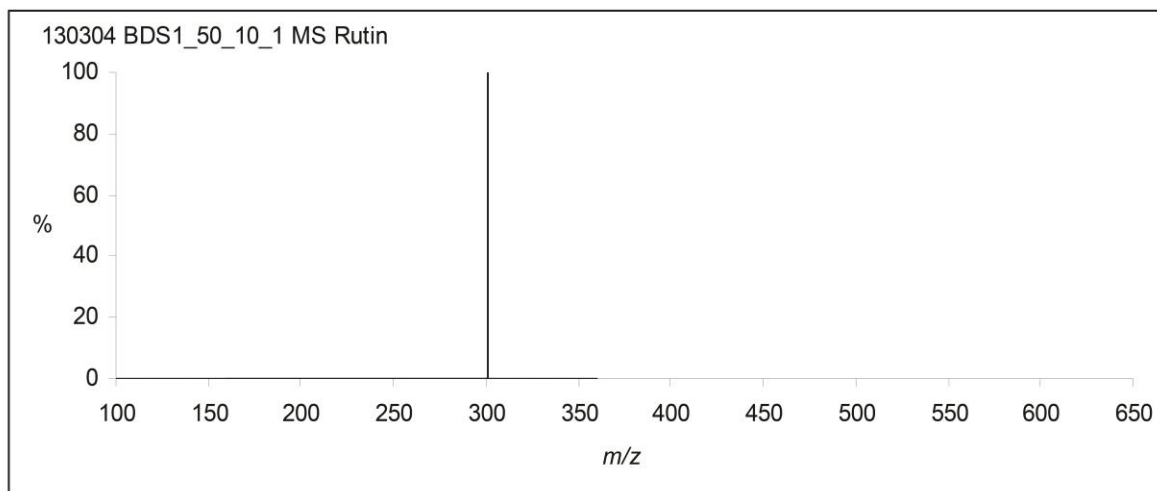


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55 Supplementary Figure S2. Obtained chromatograms for the flavonoids extracts of horse chesnut seed

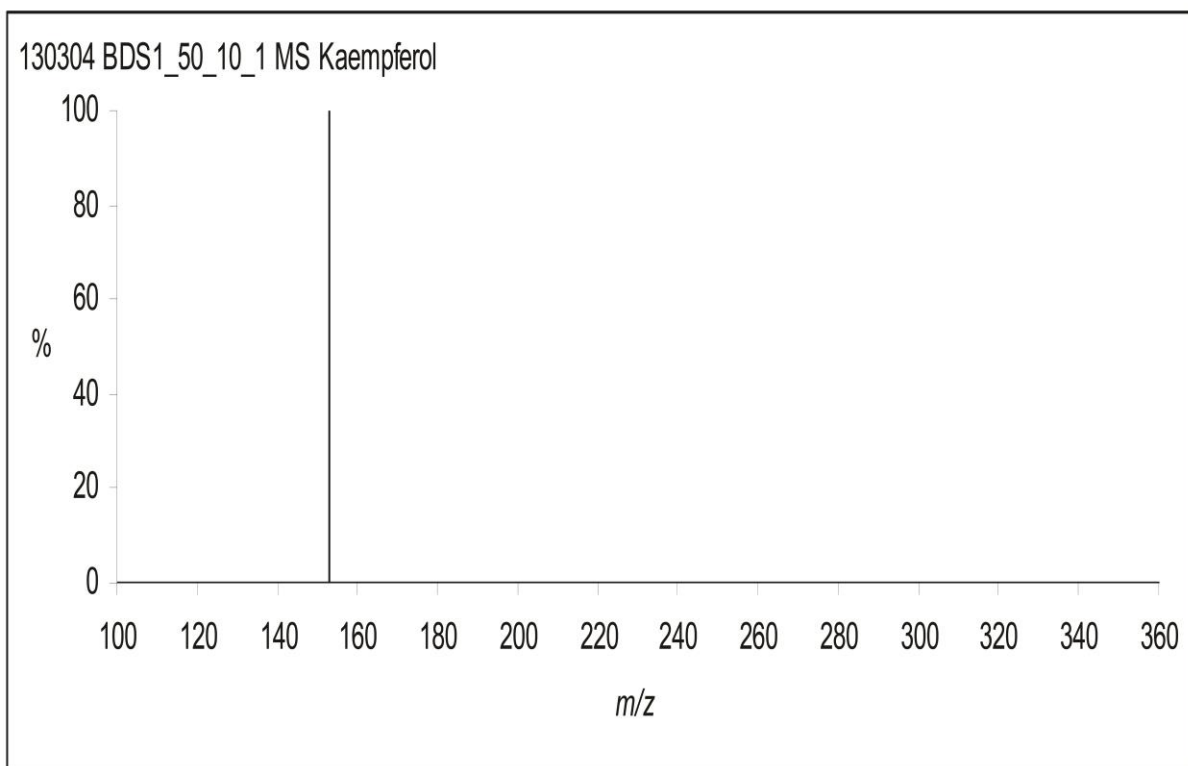
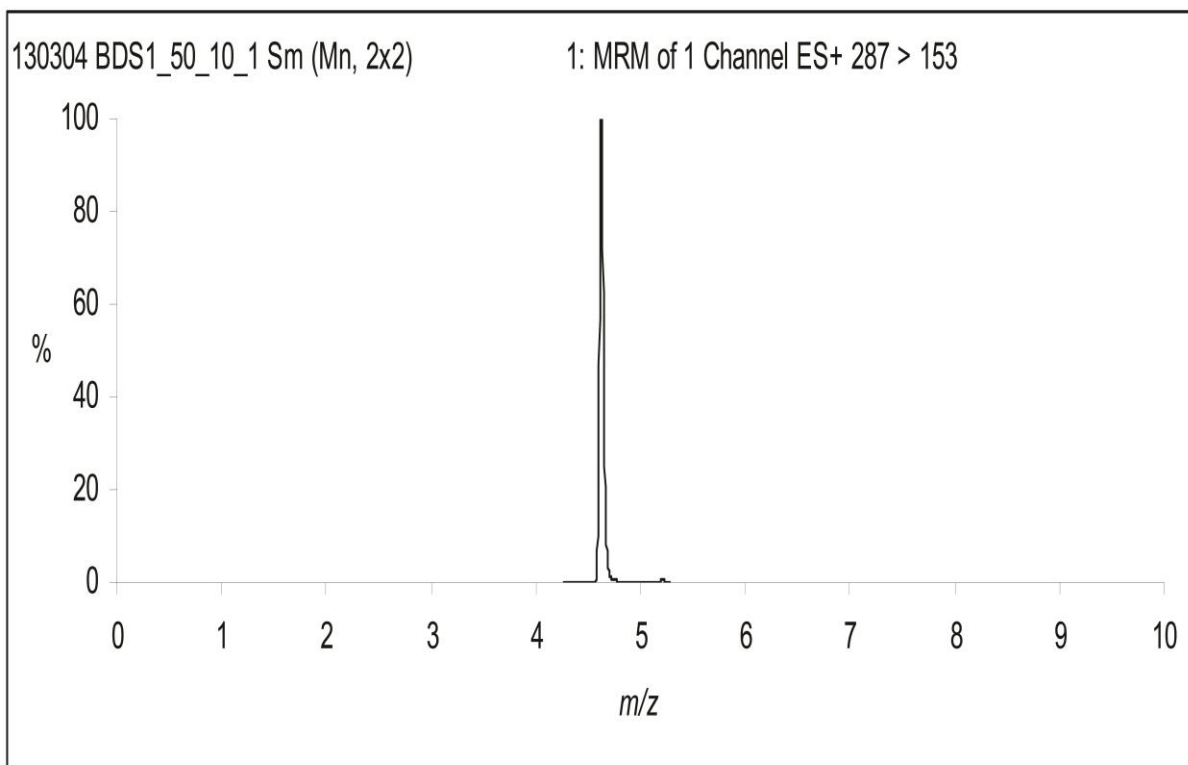


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59 Supplementary Figure S3. Obtained chromatograms for the flavonoids extracts of horse chesnut seed



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