Supporting Information

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Determination of phenolic content of Hypericum aucheri Jaub. & Spach by LC-HRMS and its antioxidant capacity

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S1 : Materials and Methods

S1.1: Chemicals

Ascorbic acid (≥99 %, Sigma-Aldrich), (-)-Epigallocatechin gallate (>97% TRC Canada), Fumaric acid (≥99 % Sigma-Aldrich), Chicoric acid (>97% TRC Canada), Caffeic acid (≥ 98 % Sigma-Aldrich), (+)-trans taxifolin (>97% TRC Canada), Luteolin-7-rutinoside (>97% Carbosynth limited), Vanilic acid (≥97 % Sigma-Aldrich), Luteolin 7-glucoside (>97% TRC Canada), Syringic acid (≥95 % Sigma-Aldrich), Rosmarinic acid (≥96 % Sigma-Aldrich), Apigenin 7-glucoside (>97% EDQM CS), Ellagic acid (>97% TRC Canada), Nepetin-7-glucoside (>97% Phytolab), Quercetin (≥95% Sigma-Aldrich), Herniarin (>98% Carl Roth GMBH), Naringenin (≥95 % Sigma-Aldrich), Luteolin (95% Sigma-Aldrich), Apigenin (>97% TRC Canada),Hispidulin (>97% TRC Canada), Isosakuranetin (>97% Phytolab), CAPE (Caffeic Asit Phenethyl Ester) (≥97% european pharmacopoeia reference standard), Chrysin (≥96% Sigma-Aldrich), Acacetin (>97% TRC Canada), Emodin (90% Sigma-Aldrich), (-)- caryophyllene oxide(≥99% chemika) were used.

S1.2: LOD and LOQ

The limit of detection (LOD) and the limit of quantification (LOQ) for each compound were determined according to the following equation: LOD or LOQ = κSDa/b, where κ = 3 for LOD and κ = 10 for LOQ, SDa represents the standard deviation of the intercept, and b represents the slope.

S1.3: Identification of Uncertainty Sources

The measurement uncertainty of the developed method was estimated using the same bottom-up approach. Main sources included sample weighing, calibration curve, and repeatability. The detailed evaluation process and formulas are documented in prior references. To prevent repetition, the calculation of the combined standard measurement uncertainty for target compounds in plant extracts is summarized in an equation. The expanded uncertainty was calculated by multiplying this combined value by 2, indicating a 95% confidence level. Uncertainty values for the measurement results are provided in Table S1.

$$u_{Combined} = \sqrt{(u_{standard})^2 + (u_{weighing})^2 + (u_{recovery})^2 + (u_{curve})^2}$$

$u_{combined}$: combined uncertainty
$u_{standart}$: uncertainty from purity of standard
$u_{weighing}$: weighing
$u_{recovery}$: precision
$u_{linearity}$: calibration curve

S1.4: DPPH free radical scavenging method

The free radical scavenging activity of C, Ac and MeOH extracts of *H. aucheri* were determined using the DPPH (1,1-diphenyl-2-picrylhydrazyl) assay. DPPH exhibits a strong absorbance at 517 nm in its radical form; however, upon reduction by an antioxidant or another radical species, its absorbance decreases. Briefly, a 0.1 mM DPPH solution in methanol was prepared, and 160 µL of this solution was added to 40 µL of sample solutions in methanol at concentrations of 10, 25, 50, and 100 µg/mL. The mixtures were kept in the dark for 30 min, and the absorbance was then measured at 517 nm. The synthetic antioxidants butylated hydroxyanisole (BHA) and butylated hydroxytoluene (BHT) were used as reference compounds.

S1.5: CUPRAC method

The reducing capacity of C, Ac and MeOH extracts *of H. aucheri* were evaluated using the CUPRAC method. Solutions of 1 mM DMF, 10 mM $CuCl_2$, 7.5 mM neocuproine, 1 M NH4-CH_3COO (pH 7.0), and distilled water were mixed in a 1:1:1:0.6 volume ratio. Subsequently, 180 µL of this mixture was added to the wells, followed by 25 µL of the test compounds diluted 1:20 in ethanol. The samples were incubated for 30 min at 25 °C, and the absorbance was measured at 450 nm against a reagent blank. In the assay, ethanol (EtOH) served as the negative control, whereas curcumin was used as the positive control.

Table S1: Analytical parameters of the LC–HRMS method for the phytochemicals

Compound	Ionization mode	Found *m/z*	Calculated *m/z*	Error (ppm)	Liner regresion equation	R^2	LOD/LOQ*	Recovery %
Fumaric Acid	Negative	115.0036	116.0104	3.15	y=0.02396x + 0.00414	0.998	0.21/0.70	94.5
Herniarin	Positive	177.0546	176.0468	0.94	y=5.23600 x + 0.72060	0.998	0.14/0.46	106.8
Caffeic acid	Negative	179.0340	180.0417	2.41	y=0.64270x + 0.05448	0.998	0.12/0.41	106.5
Chrysin	Positive	255.0651	254.0574	1.19	y=1.71000 + 0.22550	0.997	0.11/0.37	105.9
Apigenin	Negative	269.0455	270.0534	2.72	y=0.74000 x + 1.17100	0.999	0.15/0.50	101.3
Emodin	Negative	269.0455	270.0534	2.39	y=6.84500 x + 8.01000	0.999	0.10/0.35	104.0
Naringenin	Negative	271.061	272.0679	4.15	y=0.81220 x +0.04423	0.998	0.10/0.33	96.4
Isosakuranetin	Negative	285.0768	286.0836	1.21	y=1.36000 x + 0.15420	0.998	0.20/0.60	108.6
Acacetin	Positive	285.0757	284.0690	1.50	y=0.60420 x + 0.64820	0.996	0.20/0.62	104.3
Luteolin	Positive	287.0550	286.0472	1.91	y=0.15090 x + 0.03393	0.997	0.16/0.55	102.1
Kaempferol	Positive	287.0550	286.0472	0.91	y=0.13150 x + 0.02696	0.997	0.15/0.51	102.9
Scutellarein	Positive	287.0550	286.0472	1.44	y=0.11820 x + 0.00060	0.998	0.10/0.30	96.4
Dihydrokaempferol	Negative	287.0561	288.0628	3.80	y=0.84890x + 0.14350	0.999	0.12/0.39	102.7
(-)Epicatechin	Positive	291.0863	290.0785	3.62	y=0.08876x + 0.02122	0.998	0.17/0.55	103.4
(+)-Catechin	Positive	291.0863	290.0785	1.84	y=0.08329x + 0.00524	0.998	0.13/0.45	103.4
Hispidulin	Positive	301.0706	300.0628	1.73	y=0.53940 x + 0.13180	0.999	0.10/0.30	107.8
Rhamnocitrin	Positive	301.0706	300.0628	3.21	y=0.15590 x + 0.25980	0.999	0.14/0.46	108.4
(+)-Trans Taxifolin	Negative	303.0510	304.0578	2.97	y=0.24450x - 0.01517	0.998	0.12/0.44	102.5
Quercetin	Positive	303.0499	302.0421	1.30	y=0.06643 x - 0.01097	0.984	0.33/1.10	108.1
(-)-Epigallocatechin	Positive	307.0812	306.0734	3.11	y=0.04801x - 0.00370	0.998	0.16/0.56	104.7
3-O-Methylquercetin	Negative	315.0510	316.0578	2.95	y=0.08915 x +0.341	0.997	0.23/0.77	112.4
Nepetin	Negative	315.0510	316.0578	2.76	y=0.79200 x + 0.00364	0.998	0.10/0.33	92.9
Penduletin	Positive	345.0968	344.0891	1.58	y=3.387 x + 1.2	0.999	0.06/0.20	101.65
Eupatilin	Positive	345.0968	302.0057	1.38	y=3.178 x + 0.9574	0.997	0.20/0.66	106.9
Rosmarinic Acid	Negative	359.0772	360.0840	4.38	y=0.09963x - 0.00258	0.999	0.06/0.20	100.8
Apigenin 7-glucoside	Positive	433.1129	432.1051	3.13	y=0.09784x + 0.00958	0.997	0.11/0.37	107.9
Kaempferol-3-O-glucoside	Positive	449.1078	448.1000	4.15	y=0.07406x + 0.01329	0.996	0.20/0.60	113.75
Orientin	Positive	449.1078	448.1000	3.83	y=0.05944x - 0.00002	0.997	0.10/0.32	104.9
Quercitrin	Positive	449.1078	448.1000	4.78	y=0.07564 x + 0.00577	0.99	0.12/0.38	116.4
(-)-Epigallocatechin gallate	Negative	457.0776	458.0844	2.65	y=0.03253x + 0.00072	0.997	0.10/0.30	100.3
Myricitrin	Positive	465.1027	464.0949	3.11	y=0.03812x + 0.00508	0.998	0.12/0.41	102.4
Hyperoside	Positive	465.1027	464.0949	3.01	y=0.03837x + 0.00357	0.998	0.11/0.40	103.7
Hederagenin	Negative	471.3479	472.3547	9.23	y=0.0031x + 0.009	0.994	0.90/2.90	96.5
Nepetin-7-glucoside	Negative	477.1038	478.1106	4.39	y=0.11530x + 0.00497	0.999	0.06/0.20	103.6
Quillaic acid	Negative	485.3272	486.3340	3.01	y=0.29780 x + 0.01883	0.999	0.08/0.26	102.8
Naringin	Negative	579.1719	580.1787	2.84	y=0.05422x - 0.00255	0.999	0.10/0.30	105.9
Luteolin-7-rutinoside	Negative	593.1511	594.1579	1.43	y=0.07102x - 0.01863	0.998	0.20/0.60	99.5
Hesperidin	Negative	609.1824	610.1892	2.82	y=0.03076x - 0.00253	0.999	0.11/0.36	105.7
Rutin	Negative	609.1461	610.1528	4.47	y=0.03758x + 0.00045	0.999	0.10/0.31	103.3
Verbascoside	Negative	623.1981	624.2049	3.59	y=0.05182x + 0.00063	0.998	0.13/0.41	108.7

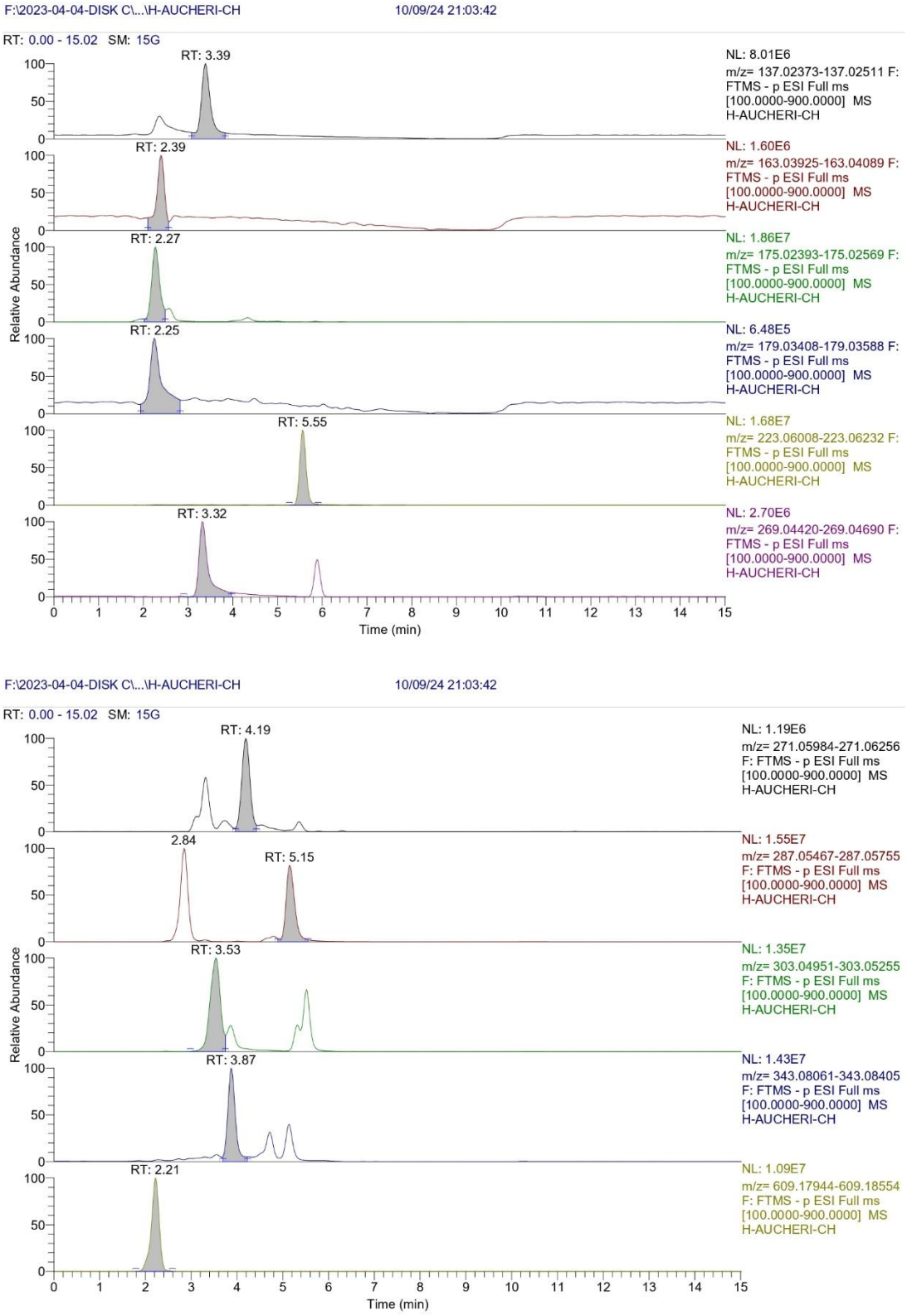

Figure S1: The LC-HRMS Chromatogram of Quantified Compounds of the Chloroform Extract of *H. aucheri*.

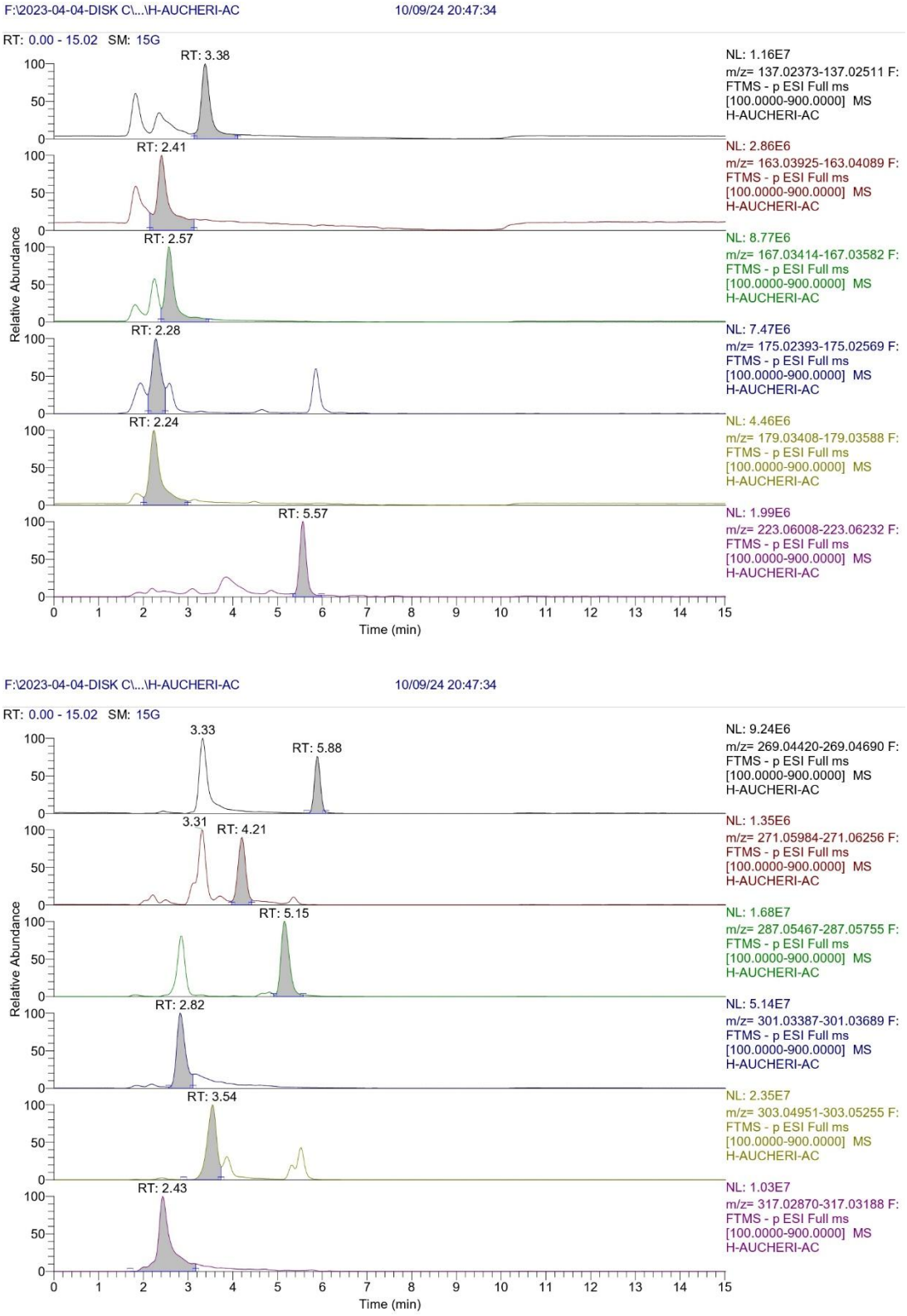

Figure S2: The LC-HRMS Chromatogram of Quantified Compounds of the Acetone Extract of *H. aucheri*

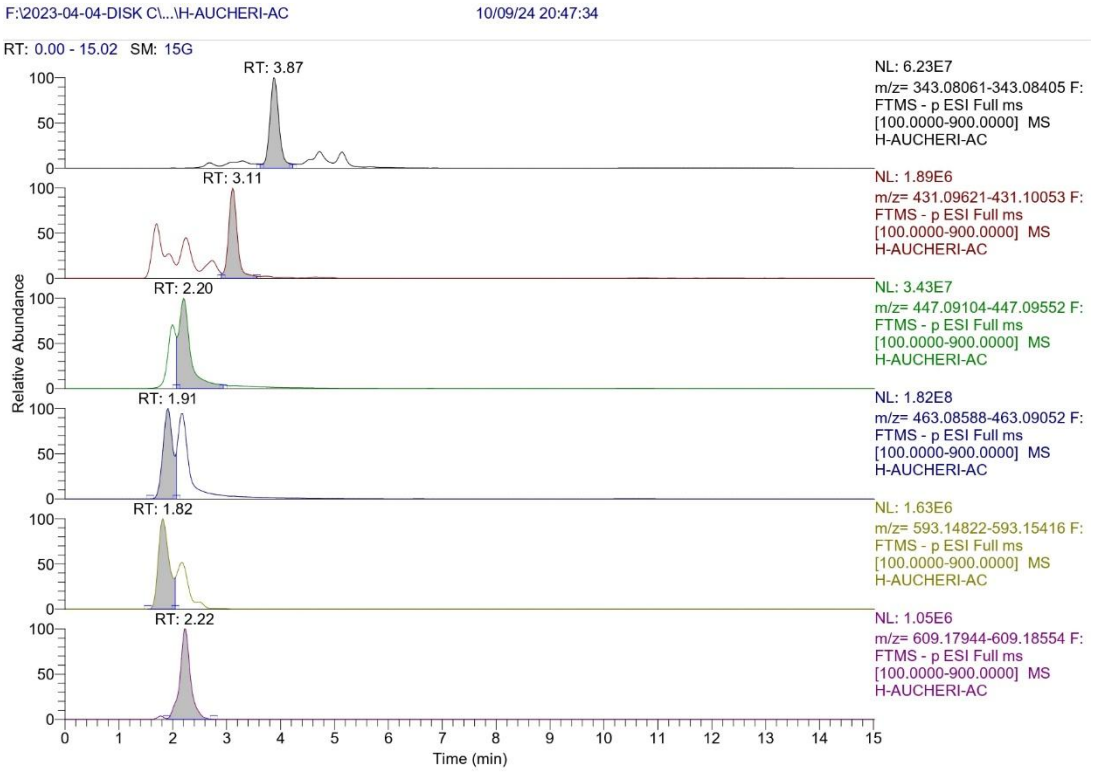

Figure S2 : Continued

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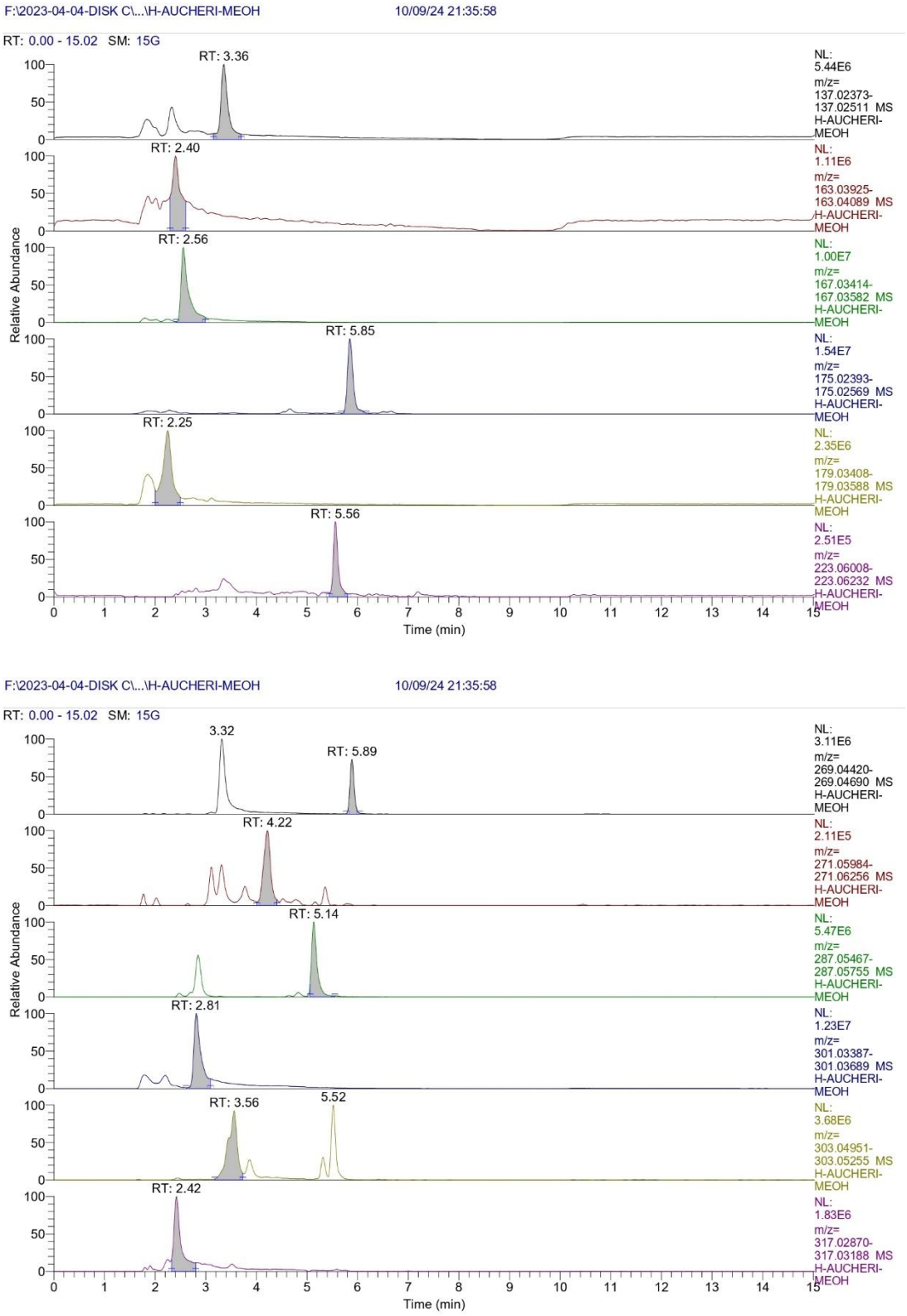

Figure S3: The LC-HRMS Chromatogram of Quantified Compounds of the Methanol Extract of *H. aucheri*.

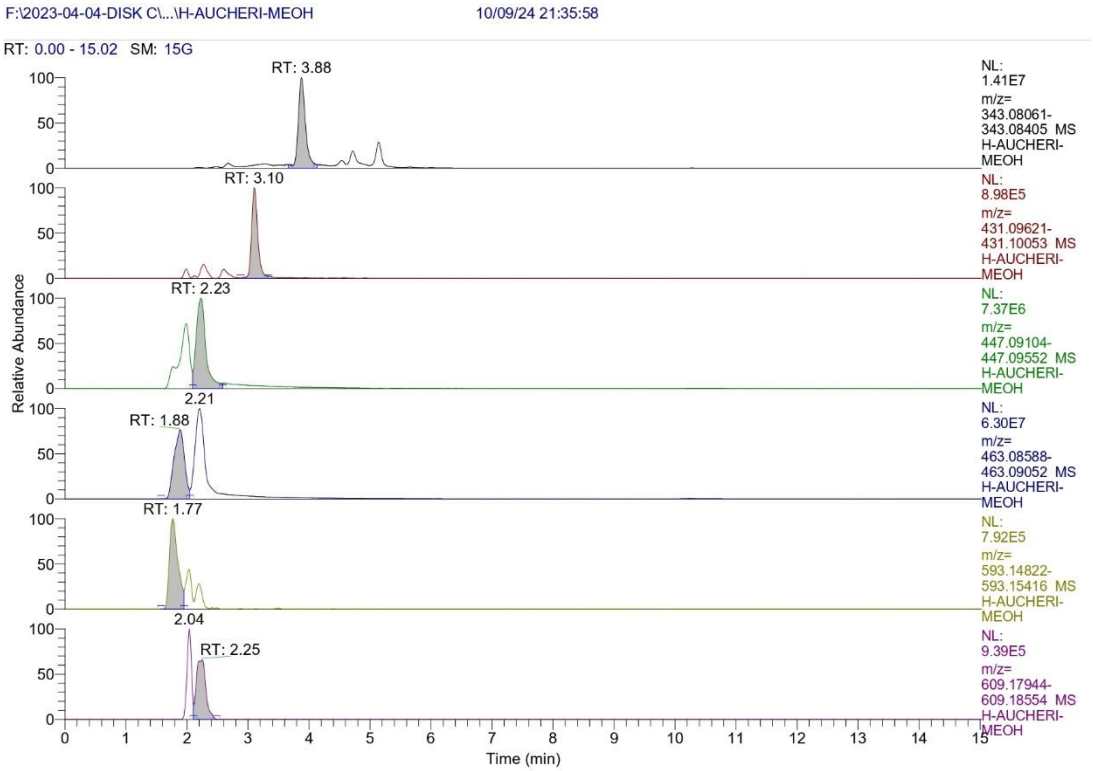

Figure S3 : Continued