International Journal of *Molecular Sciences*

MDPI

Supplementary Materials

Iron-Responsive Inhibition of Growth and Post-Antibiotic Regrowth of Uropathogenic *Escherichia coli* by Aqueous Plant Extracts

Marcin Równicki *

Microbiota Lab, Department of Pharmaceutical Microbiology and Bioanalysis, Medical University of Warsaw, Warsaw, Poland; marcin.rownicki@wum.edu.pl

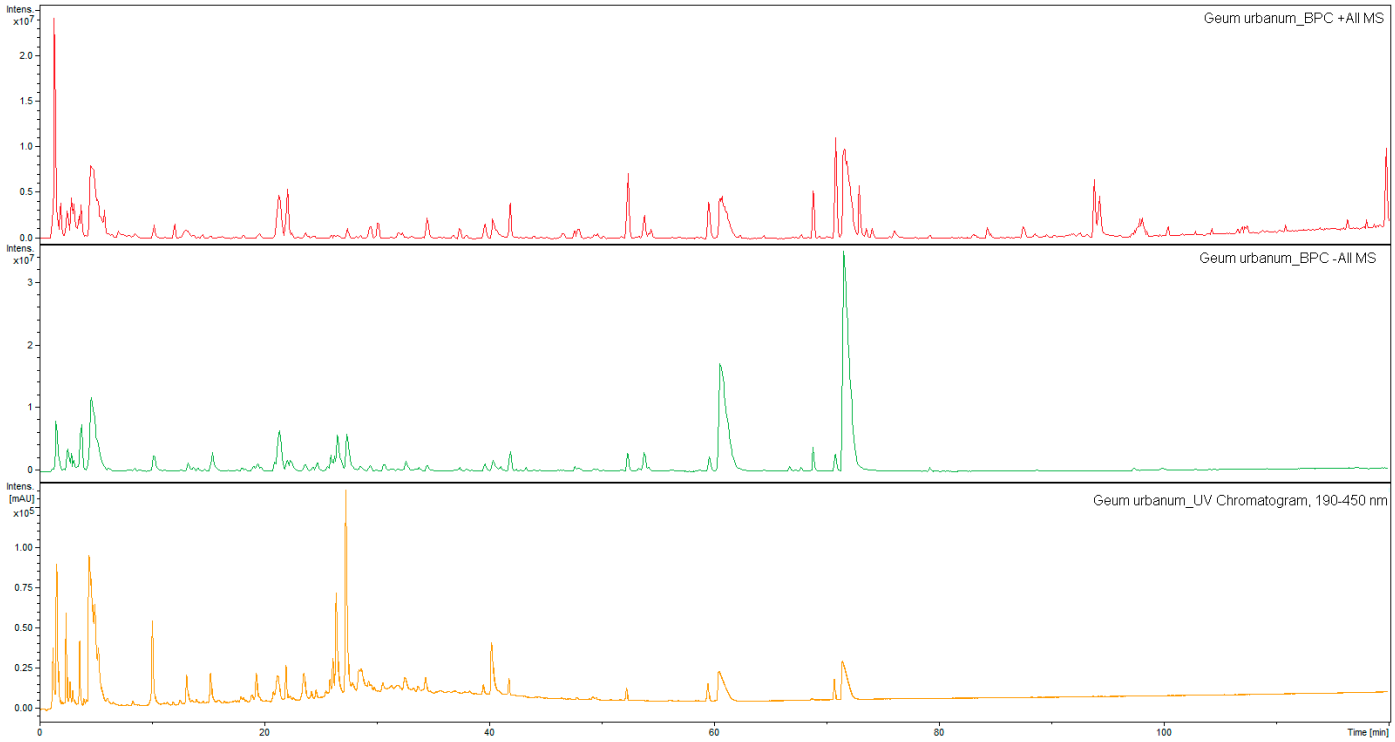

Figure S1 HPLC-MS *(*positive and negative ionization mode*)*, and HPLC–UV *(190-450 nm)* chromatograms of *G. urbanum* aqueous extract.

Table S1 UV–Vis and MS data of compounds tentatively identified in *G. urbanum* aqueous extract. Compound annotations are tentative and based on UV–Vis and MS^n data compared with published literature. The chromatographic data provide qualitative chemical profiles.

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
1	Unknown	2.8	217	497	**301**	499	–
2	Unknown	3.1	221, 286	355	**337**	357	**339**, 293, 321
3	Unknown	4.6	314	183, 367	367: **183**; 183: **139**	185	–

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
4	Gallic acid	5.0	214, 270	169	–	171	–
5	Unknown	6.1	214, 270	331	**271**	–	–
6	Galloyl–ellagic acid derivative	6.2	215, 269	649	**301**, 497	–	–
7	Unknown	8.5	214, 272	347	**195**, 329, 129	–	–
8	Digalloylhexose	13.7	213, 276	483	**331**, 169, 313	–	–
9	Flavano-ellagitannin	15.4	210, 280	527	**382**, 289	1057	–
10	Flavano-ellagitannin	19.1	210, 280	527	**382**, 505	–	–
11	Procyanidin B-type dimer I	21.4	277	577	**407**, 425, 289, 451	579	**427**, 409, 301, 291
12	Catechin	22.1	278	289, 579	**245**, 205, 203	291	**123**, 139, 165, 151
13	Procyanidin B-type trimer	23.6	280	865	**695**, 407, 577, 739, 451, 425	867	**579**, 577, 409, 407
14	Ellagitannin (casuarinin/stachyurin-type)	26.2	220, 268	935, 467	935: **917**, 857, 875; 467: **458**, 436	–	–
15	Flavano-ellagitannin (stenophyllanin A-type)	26.5	220, 280	603	**458**	1209, 605	–
16	Dimeric ellagitannin	27.3	200, 280	935, 467	935: **633**, 917; 467: **275**, 301, 458, 382	937, 617, 635	–
17	Procyanidin B-type tetramer	28.6	280	576	**289**, 407, 863, 500	–	–
18	Procyanidin B-type	29.4	280	577	**407**, 425, 289, 451	579	**409**, 435, 453, 427
19	Flavano-ellagitannin	30.6	n.d.	612	**458**, 301, 289, 935	–	–
20	Ellagitannin	31.3	n.d.	508	**423**, 432, 303, 289, 727	1019	–
21	Valoneic acid dilactone	31.8	254, 364	469	**425**	–	–
22	Procyanidin B-type	32.1	201, 278	720	**865**, 577, 739	–	–
23	Flavano-ellagitannin (stenophyllanin A-type)	32.6	220, 280	603	**458**	1209	–
24	Ellagitannin	33.8	272	588	**301**, 560, 512	–	–
25	Procyanidin B-type dimer gallate	34.5	277	729	**577**, 407, 559, 425	731	**409**, 271, 579

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
26	Unknown	37.4	259	441	**331**, 397	443	**263**, 233, 245, 281
27	Methylellagic acid hexoside	39.6	248, 365	477	**315**	479	**317**
28	Ellagic acid	40.4	252, 366	301, 603	603: **301**	303	–
29	Methylellagic acid derivative	41.9	246, 366	491	**328**, 313, 476	510, 515, 331	510: **331**
30	Sulfated methylellagic acid derivative	43.3	245, 355	571	**491**	–	–
31	Methylellagic acid pentoside	47.9	249, 365	447	**315**	449	**317**
32	Methylellagic acid	49.4	250, 367	315	**300**	317	–
33	Tri-O-methylellagic acid hexoside	52.3	247, 366	551, 505	551: **343**	529, 524, 345	345: **313**, 330
34	Unknown	53.4	n.d.	515	**291**, 485, 455	517	–
35	Triterpenoid acid hexosyl ester	53.8	n.d.	711	**503**, 665	487, 469, 451	469: **451**, 405, 423, 433
36	Methylellagic acid derivative	59.5	246, 374	329, 659	**314**	331	**299**
37	Di-O-methylellagic acid sulfate	60.5	246, 363	409	**329**	411	**331**
38	Unknown	66.7	n.d.	539	**479**	–	–
39	Unknown	70.8	n.d.	407	**327**	–	–
40	Tri-O-methylellagic acid sulfate	71.5	247, 361	423	**343**	425	**345**
41	Unknown	79.2	n.d.	517	**455**, 440, 473	501	**483**, 455, 437

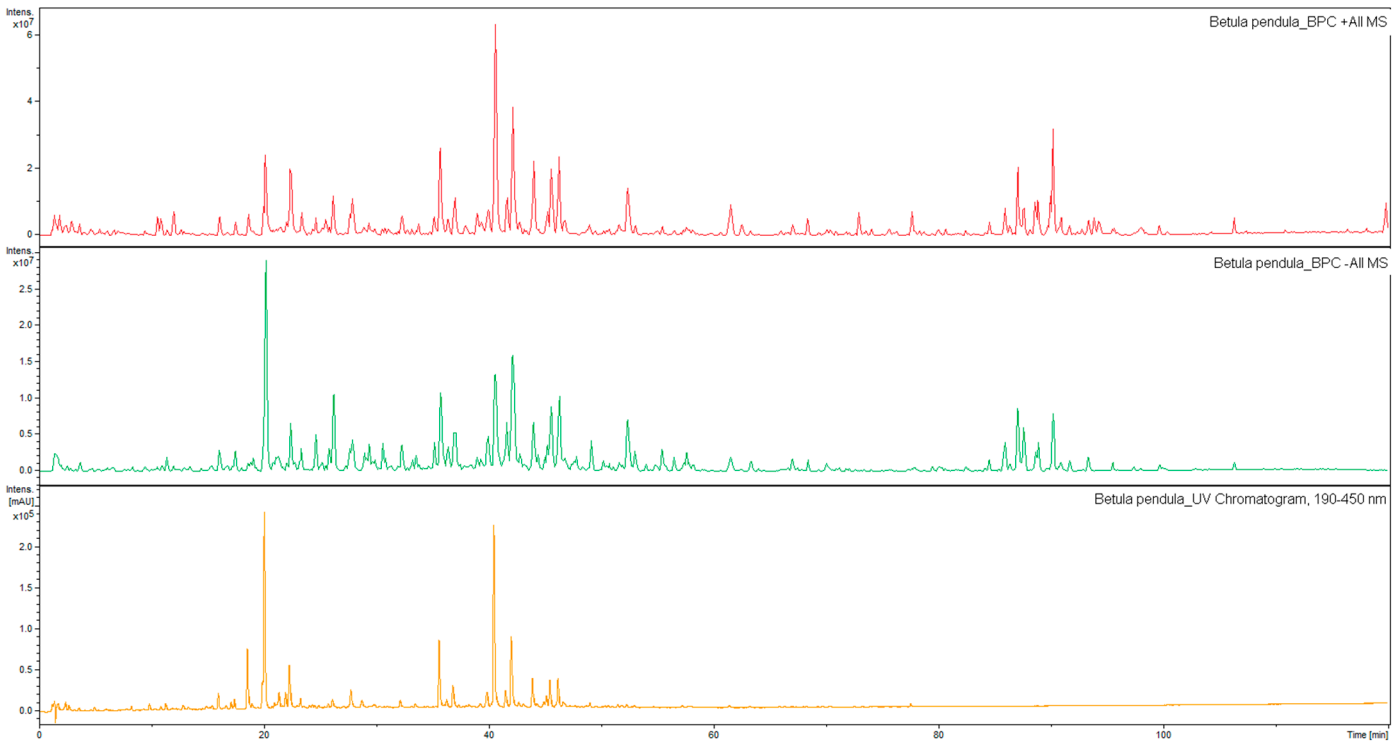

Figure S2 HPLC-MS (positive and negative ionization mode), and HPLC–UV (190-450 nm) chromatograms of *B. pendula* aqueous extract.

Table S2 UV–Vis and MS data of compounds tentatively identified in *B. pendula* aqueous extract. Compound annotations are tentative and based on UV–Vis and MS^n data compared with published literature. The chromatographic data provide qualitative chemical profiles.

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
1	Protocatechuic acid hexoside	11.4	n.d.	315, 631	**153**	–	–
2	Neochlorogenic acid	16.0	216, 323	353, 707	**191**, 179	–	–
3	Unknown	17.5	219, 275	345	**299**	318	**247**, 187, 205, 265
4	p-Coumaroylquinic acid	20.0	n.d.	337, 675	**163**	–	–
5	Dihydroxypropiophenone hexoside	20.1	218, 276	327, 655	**147**	329, 351	329: **167**
6	Procyanidin B-type dimer	21.3	283	577	**407**, 425, 289, 451	–	–
7	(Epi)catechin	22.1	n.d.	289, 579	**245**, 205, 203, 179	–	–
8	Chlorogenic acid	22.4	216, 324	353, 707	**191**	355	**163**
9	Unknown	23.3	226, 276	395	**349**	–	–
10	Unknown	28.9	310	461	**415**, 311, 149	–	–

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
11	Unknown	32.3	305	377	**331**, 179	–	–
12	Unknown	33.6	295	343, 687	**269**, 271, 283, 257	–	–
13	Myricetin hexoside	35.7	259, 355	479	**317**	481	**319**
14	Myricetin hexuronide	36.9	260, 354	493	**317**	495	**319**
15	Myricetin pentoside	40.0	261, 353	449	**317**	451	**319**
16	Quercetin hexoside	40.6	255, 353	463, 927	**301**	465	**303**
17	Rutin	40.9	n.d.	609	**301**	–	–
18	Quercetin hexuronide	42.1	256, 353	477, 955	**301**	479	**303**
19	Quercetin pentoside	44.0	255, 354	433	**301**	435	**303**
20	Quercetin malonylhexoside	45.2	258, 353	549, 505	**505**	551	**303**
21	Kaempferol hexuronide	46.8	264, 355	461	**285**	463	**287**
22	Di-O-caffeoylquinic acid	46.9	n.d.	515	**353**	–	–
23	Unknown	49.1	311	429	**249**, 205, 161, 179, 385	–	–
24	Kaempferol deoxyhexoside	51.6	263, 325	431	**285**	433	**287**
25	p-Coumaroyl-caffeoylquinic acid	52.4	n.d.	499	**369**, 353, 337	501	**483**, 167, 317
26	Quercetin acetylhexuronide	53.0	256, 356	519	**301**	521	**303**
27	Quercetin acetyldeoxyhexoside	57.4	254, 319	489	**301**, 447	–	–
28	Methylated flavonoid hexoside	61.5	275, 331	521, 475	521: **475**, 313	477	**315**
29	Dammarane-type triterpenoid	86.0	n.d.	537	**491**	457, 439, 421	457: **439**, 421

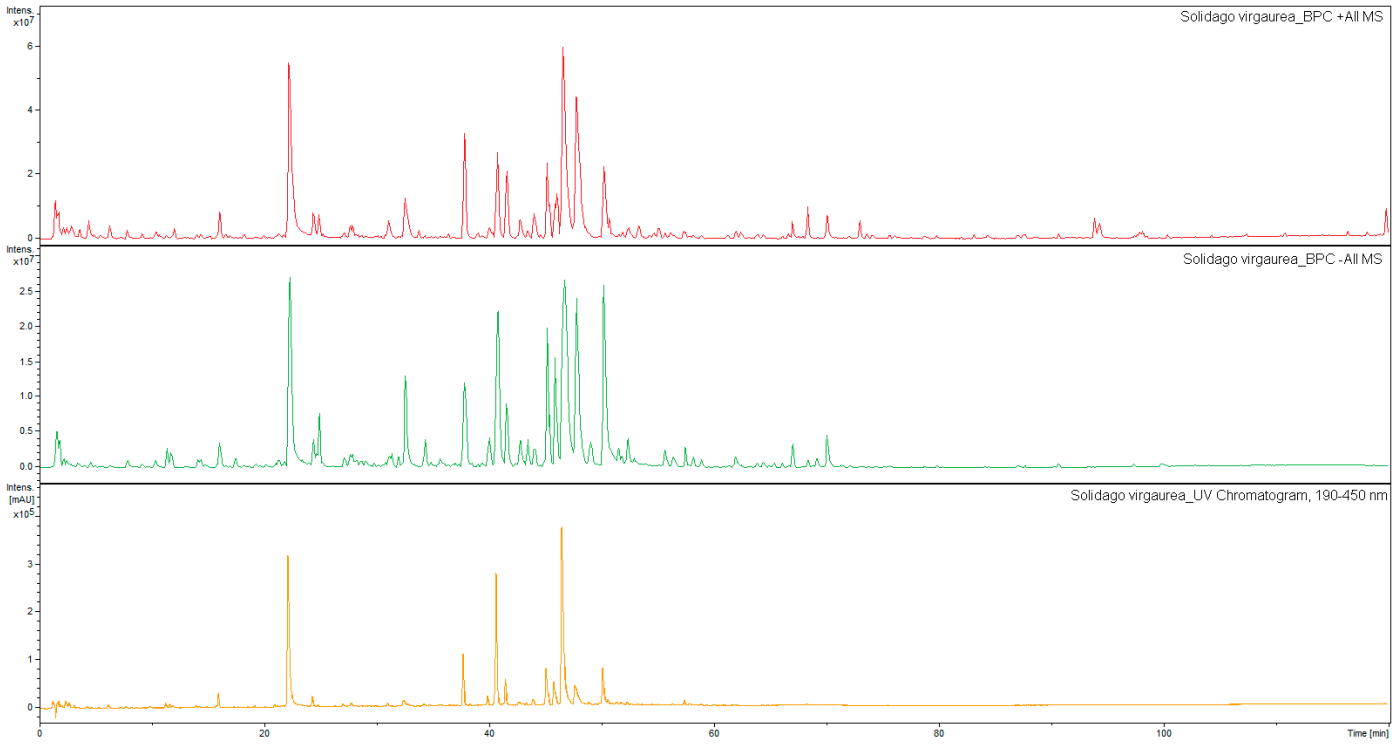

Figure S3 HPLC-MS (positive and negative ionization mode), and HPLC–UV (190-450 nm) chromatograms of *S. virgaurea* aqueous extract.

Table S3 UV–Vis and MS data of compounds tentatively identified in *S. virgaurea* aqueous extract. Compound annotations are tentative and based on UV–Vis and MS^n data compared with published literature. The chromatographic data provide qualitative chemical profiles.

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
1	Neochlorogenic acid	16.0	216, 323	353, 707	**191**, 179	355	**163**
2	Protocatechuic acid pentosyl-hexoside	17.5	n.d.	447	**315**, 152, 271	–	–
3	Chlorogenic acid	22.3	216, 324	353, 707	**191**	355	**163**
4	Caffeoylquinic acid isomer	27.1	314	353, 707	**191**	–	–
5	p-Coumaroylquinic acid	27.9	225, 311	337, 675	**191**	–	–
6	Feruloylquinic acid	31.2	216, 324	367	**191**	–	–
7	Unknown	34.3	247	583	**297**	–	–
8	Leiocarposide	37.8	n.d.	613, 659	613: **345**, 451; 659: **613**	453, 291	453: **291**; 291: **273**, 197, 179
9	Quercetin hexoside	40.5	255, 353	463	**301**	–	–
10	Rutin	40.8	256, 353	609	**301**	611	**303**, 465
11	Isoquercitrin	41.6	255, 353	463	**301**	465	**303**

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
12	Leiocarposide isomer	41.6	n.d.	613	**345**, 451, 183	–	–
13	Kaempferol deoxyhexosyl-hexoside	42.9	268	593	**285**, 255	–	–
14	Quercetin pentoside	44.0	255, 354	433	**301**	435	**303**
15	Kaempferol-hexoside derivative	44.1	262, 350	447	**285**, 255	449	**287**
16	Syringaresinol hexoside	45.0	n.d.	579	**417**	–	–
17	Kaempferol derivative	45.1	265, 343	593	**285**	595	**287**, 449
18	Unknown	45.3	n.d.	549	**505**, 387	–	–
19	Di-O-caffeoylquinic acid derivative	45.9	215, 323	515	**353**, 173, 335, 179	517	**499**
20	Protocatechuic acid dihexoside	46.0	n.d.	477	**315**, 433	–	–
21	Kaempferol derivative	46.1	264, 365	447	**285**	449	**287**
22	Isorhamnetin deoxyhexosyl-hexoside	46.3	n.d.	623	**315**, 300	–	–
23	Di-O-caffeoylquinic acid derivative	46.6	217, 325	515, 1031	1031: **515**, 353	–	–
24	Unknown	49.0	215, 323	493	**331**	–	–
25	Di-O-caffeoylquinic acid derivative	50.2	216, 325	515	**353**, 203, 173, 299	517	517: **499**; 499: **163**, 319
26	p-Coumaroyl-caffeoylquinic acid	52.3	218, 315	499	**353**, 337	–	–
27	Di-O-caffeoylquinic acid derivative	56.4	217, 319	515	**353**	–	–

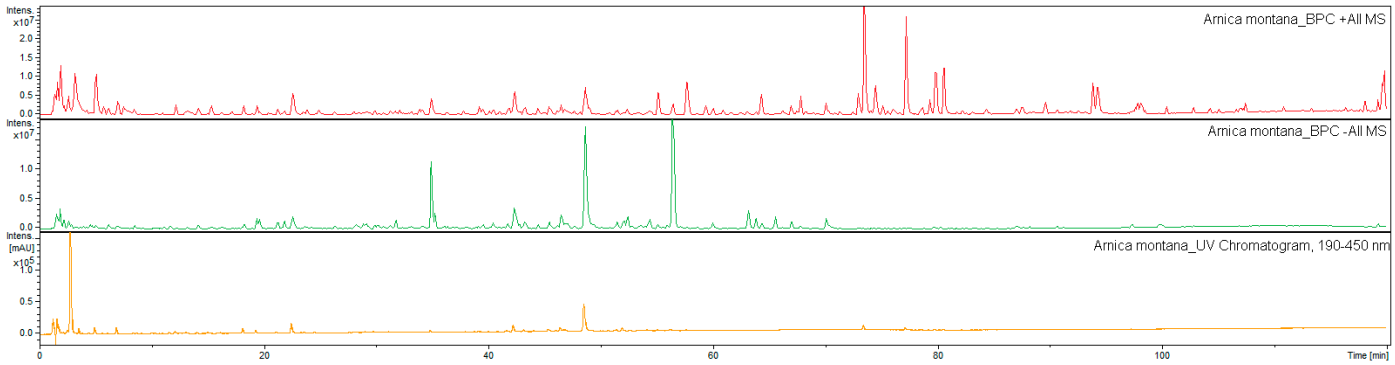

Figure S4 HPLC-MS (positive and negative ionization mode), and HPLC–UV (190-450 nm) chromatograms of *A. montana* aqueous extract.

Table S4 UV–Vis and MS data of compounds tentatively identified in *A. montana* aqueous extract. Compound annotations are tentative and based on UV–Vis and MS^n data compared with published literature. The chromatographic data provide qualitative chemical profiles.

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
1	Uridine	3.6	261	243	**200**, 110, 153, 152	–	–
2	Guanosine	7.0	253	282	**150**	284	**152**
3	Protocatechuic acid hexoside	11.7	n.d.	315	**153**, 108	–	–
4	Syringic acid hexoside	14.1	274	405, 359	405: **359**, 197	–	–
5	Neochlorogenic acid	16.2	323	353	**191**, 179	–	–
6	Caffeoylquinic acid derivative	19.4	217, 327	439	**233**, 395	441	**423**, 397
7	Chlorogenic acid	22.6	216, 324	353, 707	**191**	355	**163**
8	Unknown	29.1	265	583	**375**, 327, 537	561, 556	–
9	Feruloylquinic acid	31.3	238, 323	367	**191**	–	–
10	Unknown	31.8	n.d.	473	**427**, 247, 383, 179	–	–
11	Coumaroylhexose	33.1	n.d.	371, 325	371: **163**, 325, 135	–	–
12	Unknown	34.2	n.d.	473	**427**, 179	–	–
13	Unknown	35.2	n.d.	525, 481	525: **481**; 481: **434**, 302, 301	–	–
14	Unknown	39.5	217, 276	419	**373**, 160, 332, 179	–	–
15	Quercetin hexoside	41.8	256, 352	463	**301**	465	**303**

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
16	Patuletin hexuronide	43.2	259, 350	507	**331**, 316	509	**333**
17	Quercetin malonylhexoside	45.4	256, 356	549, 505	549: **505**; 505: **301**, 463	551	**303**
18	Kaempferol hexoside	46.2	264, 342	447	**285**	–	–
19	Di-O-caffeoylquinic acid	46.5	217, 327	515	**353**, 191	–	–
20	Kaempferol hexuronide	46.9	264, 361	461	**285**	463	**287**
21	Dicaffeoylquinic acid derivative	48.6	216, 328	601	**395**, 515, 557, 439, 233, 353	603, 620, 625	**423**, 585
22	Unknown	51.5	217, 266	605	**561**, 357	–	–
23	p-Coumaroyl-caffeoylquinic acid derivative	54.0	220, 316	585	**395**, 541, 233, 499	–	–
24	Unknown	56.4	332	609	**565**	628, 633	628: **247**, 347, 285, 329
25	Unknown	63.9	n.d.	609	**565**	628, 633	–
26	Unknown	65.5	245, 328	565	**463**, 481, 301	584, 589	–

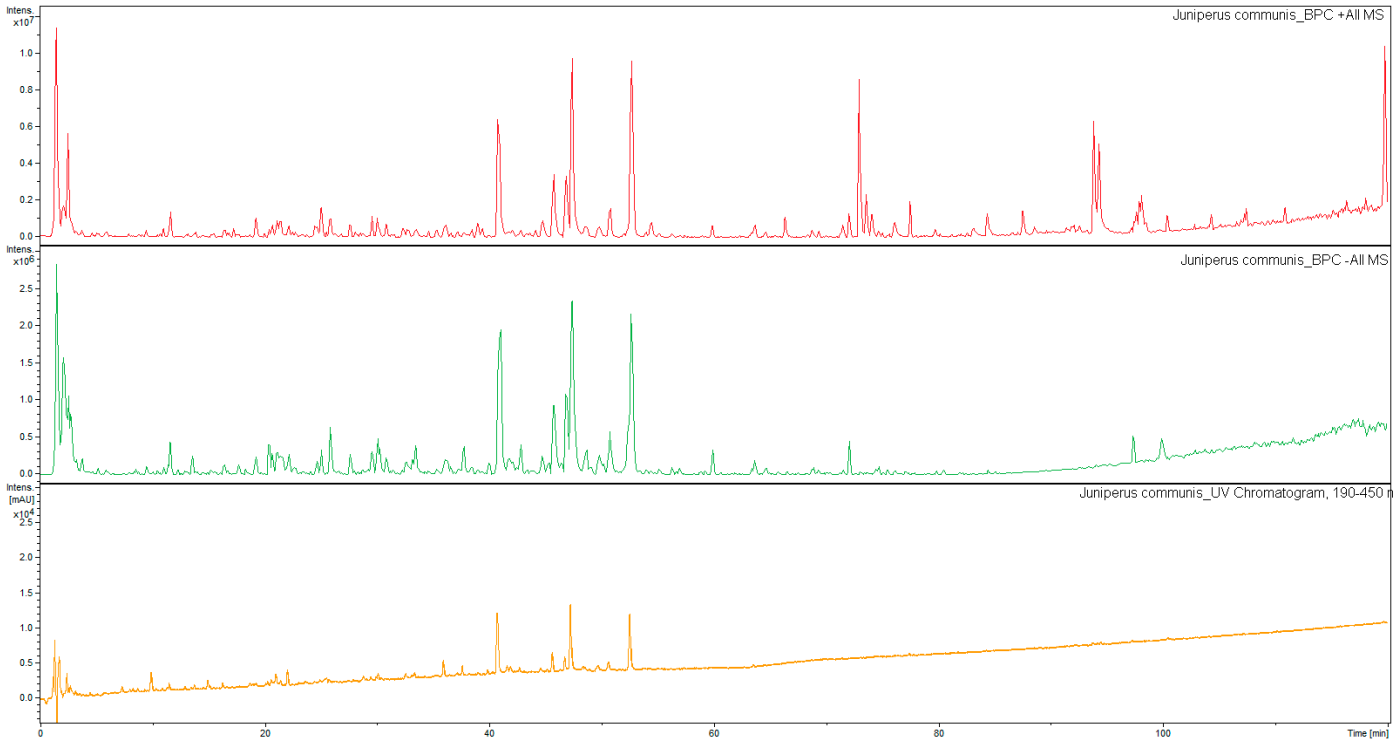

Figure S5 HPLC-MS (positive and negative ionization mode), and HPLC–UV (190-450 nm) chromatograms of *J. communis* aqueous extract.

Table S5 UV–Vis and MS data of compounds tentatively identified in *J. communis* aqueous extract. Compound annotations are tentative and based on UV–Vis and MS^n data compared with published literature. The chromatographic data provide qualitative chemical profiles.

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
1	Citric acid	2.5	n.d.	191	**111**, 173	–	–
2	Protocatechuic acid hexoside	11.4	222, 276	315	**153**, 109	–	–
3	Unknown	11.5	223, 278	405	**359**, 179	–	–
4	Protocatechuic acid dihexoside	13.5	n.d.	477	**315**, 153	–	–
5	Procyanidin B-type dimer	21.1	n.d.	577	**407**, 425, 289, 451	–	–
6	(Epi)catechin	22.1	n.d.	289, 579	**245**, 205	–	–
7	Sinapic acid hexoside	25.8	n.d.	431, 385	431: **385**, 223	–	–
8	Unknown	33.4	n.d.	525, 571	525: **507**, 383, 179, 165	–	–
9	Gossypetin glycoside	36.1	271, 360	611	**317**, 479, 449	613	**319**, 481, 451

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
10	Methoxyflavonol dihexoside	39.9	262, 360	655	**331**, 493, 316	–	–
11	Hypolaetin derivative	40.9	276, 337	463	**301**	465	**303**
12	Rutin	41.0	255, 360	609	**301**	611	**303**, 465, 449
13	Luteolin/kaempferol hexoside	42.0	348	447	**285**	–	–
14	Hypolaetin-type flavonoid pentoside	44.7	277, 340	433	**301**	435	**303**
15	Apigenin hexoside	46.8	266, 336	431	**269**	433	**271**
16	Hypolaetin-type flavonoid pentoside	47.3	274, 340	433	**301**	435	**303**
17	Quercetin hexoside	50.7	269, 351	463	**301**	465	**303**
18	Isoscutellarein derivative	52.6	275, 304	417	**285**	419	**287**
19	Methoxyflavone pentoside	53.9	n.d.	447	**315**	–	–
20	Apigenin pentoside	55.1	268, 337	401	**269**	–	–
21	Biflavone	74.4	273, 338	537	**375**, 443, 417, 399	–	–

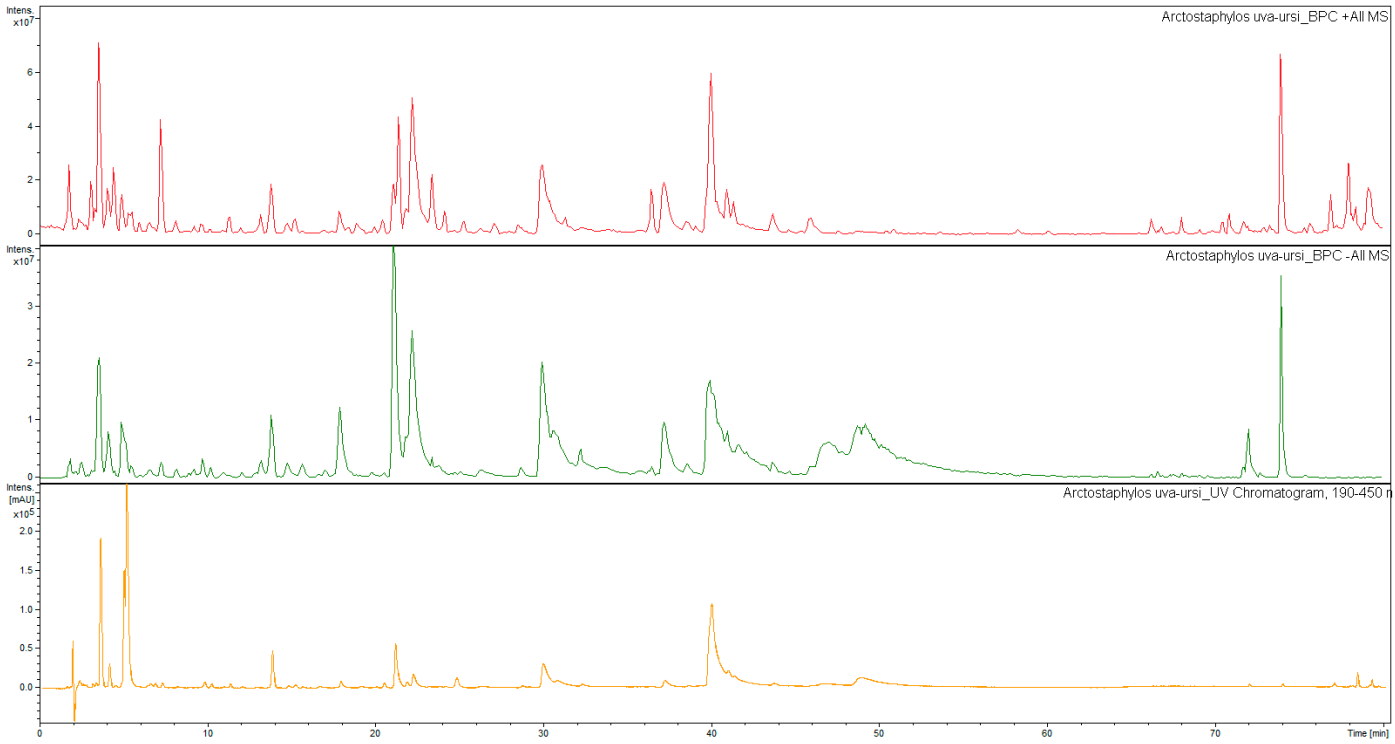

Figure S6 HPLC-MS (positive and negative ionization mode), and HPLC–UV (190-450 nm) chromatograms of *A. uva-ursi* aqueous extract.

Table S6 UV–Vis and MS data of compounds tentatively identified in *A. uva-ursi* aqueous extract. Compound annotations are tentative and based on UV–Vis and MS^n data compared with published literature. The chromatographic data provide qualitative chemical profiles.

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
1	Sucrose	1.8	n.d.	387	–	360,	**325**, 289, 343, 163, 145, 127
2	Arbutin	3.6	226, 283	317, 543, 271	317: **271**; 543: **271**	290,	**163**, 180, 201, 145
3	Hydroquinone dihexoside	4.4	283	479, 433	479: **433**; 433: **323**, 221	452,	**325**, 271, 163, 289
4	Gallic acid	5.1	224, 268	169	–	171	–
5	Galloyl dihexoside isomer	9.2	215, 273	493	**271**, 313, 331	512,517	**333**, 476, 369, 315
6	Unknown	10.1	214, 272	243, 487	487: **243**	245, 267	**153**, 227
7	Digalloylhexose isomer	10.9	214, 272	483	**331**, 169, 313	485, 507, 467	–
8	Galloylshikimic acid	12.0	213, 273	325	**169**	327, 349	**153**, 309, 171

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
9	Picein	13.8	264	343, 297, 595	343: **135**, 297	299, 316,321, 619	299: **137**
10	Galloylarbutin isomer	15.6	215, 275	423	**313**, 169	425, 442,447	–
11	Methyl gallate	16.5	215, 270	183	–	185	–
12	Procyanidin B-type dimer	19.0	277	577	**425**, 407, 451, 289	579	**427**, 409, 291, 289
13	(Epi)catechin	20.5	277	289	**245**, 205, 203	291	**139**, 123, 165, 273
14	Galloyl-hydroquinone derivative	22.2	216, 277	423	**261**, 151	442,	**263**, 153
15	Piceol	24.7	217, 274	135	–	137	–
16	Trigalloylhexose isomer	26.3	215, 274	635, 317	**465**, 483	654,659, 619	654: **619**, 467; 619: **449**, 237, 279
17	Galloylpicein	32.2	215, 266	449	**313**	451, 468,473	451: **315**, 297, 171, 153
18	Digalloylarbutin isomer	33.7	218, 277	575	**423**	594,599	–
19	Quercetin galloylhexoside	37.1	262, 354	615	**463**, 301	617, 639	**303**, 315
20	Isorhamnetin hexoside	38.5	251, 357	477	**315**	479, 501	–
21	Quercetin hexoside	39.9	256, 353	463	**301**	465	**303**
22	Quercetin hexoside (isoquercitrin)	41.0	251, 351	463	**301**	465, 487	–
23	Quercetin pentoside isomer I	43.6	253, 354	433	**301**	435	**303**
24	Kaempferol derivative	44.7	264, 349	593	**285**	595, 617	**287**, 449
25	Pentagalloylhexose	49.0	215, 278	939, 469	939: **787**, 769; 469: **393**, 618	958,963	**771**, 794
26	Quercetin derivative	50.8	n.d.	583	**463**, 301	585, 607	**303**, 283, 345

Peak number	Identification	Retention time [min]	UV–Vis max [nm]	MS ions (–) [m/z]	MS2 (–) [m/z]	MS ions (+) [m/z]	MS2 (+) [m/z]
27	Hexagalloylhexose	55.2	n.d.	545	**469**	–	–
28	Unknown	71.9	236	609, 293	609: **221**, 592, 293	277, 317	–
29	Unknown	73.9	236	293, 609	**249**, 193	295, 611	295: **221**, 239, 203, 175; 611: **317**
30	Unknown	76.9	234	–	–	277, 299	**221**
31	Unknown	78.0	241	–	–	294, 299, 575	294: **221**, 203; 575: **299**
32	Unknown	78.3	236	293, 521, 453	453: **317**, 385, 249	277, 299, 421	–
33	Unknown	79.2	276	521, 497, 453, 429	521: **385**, 453, 317	301, 579	579: **301**; 301: **245**, 283, 171

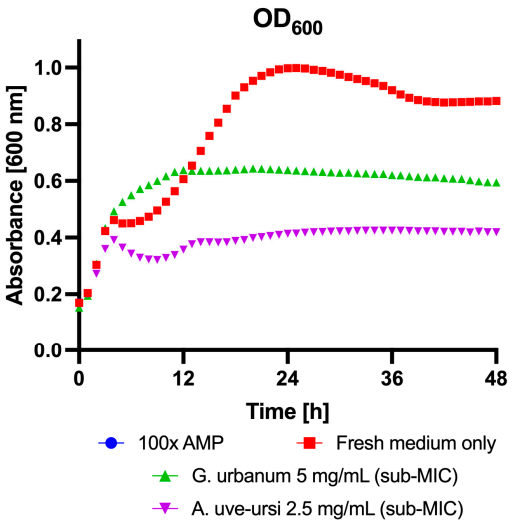

Figure S7 Effects of aqueous plant extracts on post-antibiotic regrowth of UPEC. Bacterial recovery was assessed by kinetic absorbance measurements. Error bars omitted for clarity, n=3.

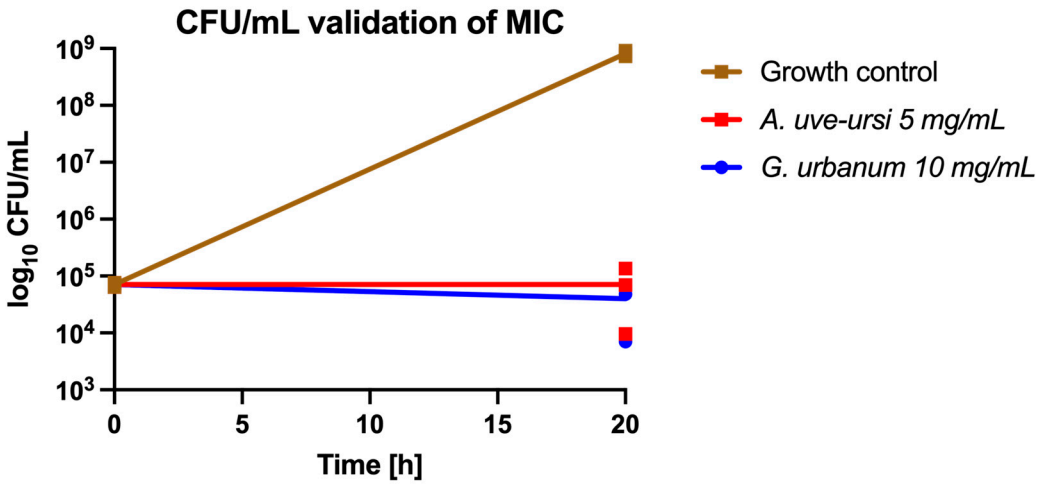

Figure S8 Viable bacterial counts assessed following 20 h of exposure to MICs of *A. uva-ursi* (5 mg/mL) and *G. urbanum* (10 mg/mL) and corresponding extract-free growth control.