

SUPPORTING MATERIAL

Antimicrobial, multidrug resistance reversal and biofilm formation inhibitory effect of *Origanum majorana* extracts, essential oil and monoterpenes

Tasneem Sultan Abu Ghazal,¹ Zsuzsanna Schelz,² Livia Vidács,¹ Nikoletta Szemerédi,³
Katalin Veres,¹ Gabriella Spengler,³ Judit Hohmann^{1,4*}

Department of Pharmacognosy, University of Szeged, 6720 Szeged, Hungary

Department of Pharmacodynamics and Biopharmacy, University of Szeged, 6720 Szeged, Hungary

Department of Medical Microbiology, Albert Szent-Györgyi Health Center and Albert Szent-Györgyi

Medical School, University of Szeged, Semmelweis utca 6, H-6725 Szeged, Hungary

Interdisciplinary Centre for Natural Products, University of Szeged, Eötvös u. 6,

H-6720 Szeged, Hungary

CONTENT

Figure S1. GC chromatogram of <i>n</i> -hexane extract of <i>Origanum majorana</i>	2
Table S1. Composition of the <i>n</i> -hexane extract of <i>Origanum majorana</i> determined by GC-MS.....	2
Table S2. Efflux pump inhibitory activity of marjoram extracts, essential oil and its constituents by real time ethidium bromide accumulation assay on <i>E. coli</i> AG100 strain	4
Table S3. Efflux pump inhibitory activity of marjoram extracts, essential oil and its constituents by real time ethidium bromide accumulation assay on <i>E. coli</i> ATCC 25922 strain	5
Table S4. Efflux pump inhibitory activity of marjoram extracts, essential oil and its constituents by real time ethidium bromide accumulation assay on <i>S. aureus</i> ATCC 25923 strain	6
Table S5. Efflux pump inhibitory activity of marjoram extracts, essential oil and its constituents by real time ethidium bromide accumulation assay on <i>S. aureus</i> MRSA ATCC 43300 strain	7

Figure S1. GC chromatogram of *n*-hexane extract of *Origanum majorana*

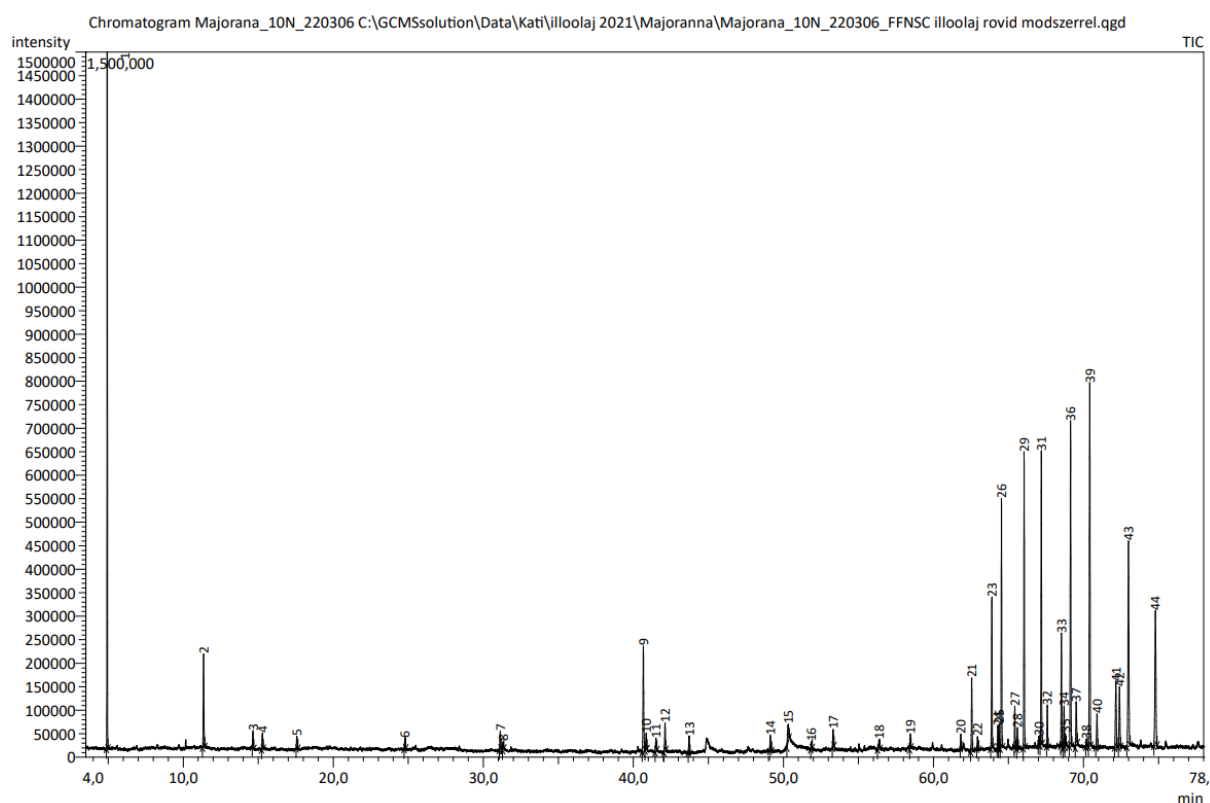


Table S1. Composition of the *n*-hexane extract of *Origanum majorana*
determined by GC-MS

Peak	Name	RI	RT (min)	Area
1	<i>n</i> -nonane	896	4.935	1043259
2	<i>trans</i>-sabinene hydrate	1099	11.357	76535
3	terpinen-4-ol	1177	14.65	22450
4	terpineol <alpha>	1192	15.277	19055
5	linalyl acetate	1245	17.574	19545
6	β-caryophyllene	1413	24.784	7847
7	spathulenol	1570	31.134	9087
8	caryophyllene oxide	1574	31.327	tr
9	neophytadiene	1829	40.669	63230
10	phytone	1835	40.865	17650
11	neophytadiene	1854	41.516	9549
12	heptadec-(4 <i>E</i>)-enol	1872	42.112	15635
13	unknown	1920	43.724	15064
14	methyl-linolenate	2089	49.125	12930

15	ethyl-linolenate	2131	50.329	tr
16	unknown	2185	51.862	tr
17	unknown	2243	53.306	32002
18	unknown	2381	56.371	15395
19	<i>n</i> -pentacosane	2487	58.455	15264
20	unknown	2652	61.821	15431
21	<i>n</i> -heptacosane	2688	62.552	83059
22	unknown	2709	62.939	34814
23	unknown	2761	63.891	165279
24	unknown	2783	64.288	21987
25	<i>n</i> -octacosane	2788	64.369	24549
26	squalene	2797	64.535	346114
27	unknown	2850	65.424	44384
28	unknown	2860	65.594	19484
29	<i>n</i> -nonacosane	2887	66.041	305629
30	unknown	2949	67.028	9378
31	<i>n</i> -pentacosane	2959	67.191	292679
32	<i>n</i> -triacontane	2984	67.582	48095
33	unknown	3041	68.528	115274
34	<i>n</i> -pentatriacontane	3052	68.703	40438
35	unknown	3056	68.783	6034
36	unknown	3078	69.137	363428
37	unknown	3100	69.513	91178
38	<i>n</i> -hexatriacontane	3141	70.204	9182
39	unknown	3154	70.414	489964
40	<i>n</i> -dotriacontane	3183	70.911	48160
41	<i>n</i> -octatriacontane	3258	72.161	100530
42	<i>n</i> -octatriacontane	3272	72.4	91952
43	<i>n</i> -tritriacontane	3308	72.996	318101
44	<i>n</i> -tetratriacontane	3415	74.789	244192

Table S2. Efflux pump inhibitory activity of marjoram extracts, essential oil and its constituents by real time ethidium bromide accumulation assay on *E. coli* AG100 strain

<i>E. coli</i> AG100	Mean	SD	RFI
Extracts	125 µg/mL		
MeOH extract	19310	98.97474	-0.1651
<i>n</i> -hexane extract	20402	1749.647	-0.1179
Essential oil	52568	1322.622	1.2729
	62.5 µg/mL		
MeOH extract	19045	103.1843	-0.1766
<i>n</i> -hexane extract	20118	121.3768	-0.1301
Essential oil	37865	1056.015	0.6372
Compounds	100 µM		
α-terpinene	23049.33	273.7158	-0.0034
γ-terpinene	22380.67	315.3321	-0.0323
terpinene-4-ol	23107	478.091	-0.0009
sabinene	22778.67	603.6094	-0.0151
sabinene hydrate	22168	53.11309	-0.0415
linalool	22026.33	419.6931	-0.0476
	50 µM		
α-terpinene	23265.67	1054.827	0.0059
γ-terpinene	22887	551.1361	-0.0104
terpinene-4-ol	22729	676.5006	-0.0173
sabinene	22180	285.454	-0.0410
sabinene hydrate	21983.67	1085.728	-0.0495
linalool	21179.67	122.4105	-0.0843
Controls			
DMSO	23128	2065.409	
<i>E. coli</i> AG100	21553.33	637.2891	-0.0681
CCCP	39281.67	156.5162	0.6985

Table S3. Efflux pump inhibitory activity of marjoram extracts, essential oil and its constituents by real time ethidium bromide accumulation assay on *E. coli* ATCC 25922 strain

<i>E. coli</i> ATCC 25922	Mean	SD	RFI
Extracts	62.5 µg/mL		
MeOH extract	22441.33	1094.717	-0.1384
<i>n</i> -hexane extract	27121	1888.161	0.0412
Essential oil	137226.7	1508.068	4.2684
	31.2 µg/mL		
MeOH extract	23364.67	544.6332	-0.1030
<i>n</i> -hexane extract	25818	1202.07	-0.0088
Essential oil	109732.3	7022.362	3.2129
Compounds	100 µM		
α-terpinene	27843.33	877.377	0.0690
γ-terpinene	26368.33	350.3574	0.0123
terpinene-4-ol	27878.67	883.148	0.0703
sabinene	32590.67	10117.96	0.2512
sabinene hydrate	26600	631.3153	0.0212
linalool	25667.67	344.6452	-0.0146
	50 µM		
α-terpinene	26550	107.5314	0.0193
γ-terpinene	27434.67	82.97188	0.0533
terpinene-4-ol	27748	692.242	0.0653
sabinene	27145.67	1438.72	0.0422
sabinene hydrate	25548.67	835.0032	-0.0191
linalool	25402.33	344.1138	-0.0248
Controls			
DMSO	26047	1128.075	
<i>E. coli</i> ATCC	26708.67	316.0575	0.0254
CCCP	78192.33	475.7534	2.0020

Table S4. Efflux pump inhibitory activity of marjoram extracts, essential oil and its constituents by real time ethidium bromide accumulation assay on *S. aureus* ATCC 25923 strain

<i>S. aureus</i> ATCC 25923	Mean	SD	RFI
Extracts	62.5 µg/mL		
MeOH extract	19704.67	1398.57	-0.17
<i>n</i> -hexane extract	22409.33	1124.71	-0.06
Essential oil	21324.00	954.24	0.19
Compounds	100 µM		
α -terpinene	25220.00	638.77	0.06
γ -terpinene	25275.67	1974.12	0.06
terpinene-4-ol	24275.33	573.93	0.02
sabinene	25065.33	570.63	0.06
sabinene hydrate	23667.67	1569.13	0.00
linalool	24364.00	1147.47	0.03
Controls			
DMSO	23751.00	389.83	
<i>S. aureus</i> ATCC	26062.00	412.66	0.10
RES	60508.67	5406.55	1.55

Table S5. Efflux pump inhibitory activity of marjoram extracts, essential oil and its constituents by real time ethidium bromide accumulation assay on *S. aureus* MRSA ATCC 43300 strain

<i>S. aureus</i> MRSA ATCC 43300	Mean	SD	RFI
Extracts	62.5 µg/mL		
MeOH extract	32845.33	1461.43	-0.52
<i>n</i> -hexane extract	95286.33	4736.54	0.39
Essential oil	65466.80	779.93	0.35
Compounds	100 µM		
α-terpinene	68390.67	1351.32	0.00
γ-terpinene	67851.33	741.31	-0.01
terpinene-4-ol	67717.33	1342.24	-0.01
sabinene	74905.00	2669.34	0.10
sabinene hydrate	87137.33	29123.07	0.27
linalool	65269.33	3227.53	-0.05
Controls			
DMSO	68370.33	894.34	
<i>S. aureus</i> MRSA 43300	72053.67	3033.87	0.05
RES	140833.33	103221.54	1.06