

SUPPLEMENTARY MATERIAL

HPTLC Bioautography-Guided Isolation of Isogeranic Acid as the Main Antibacterial Constituent of *Artemisia santonicum* Essential Oil

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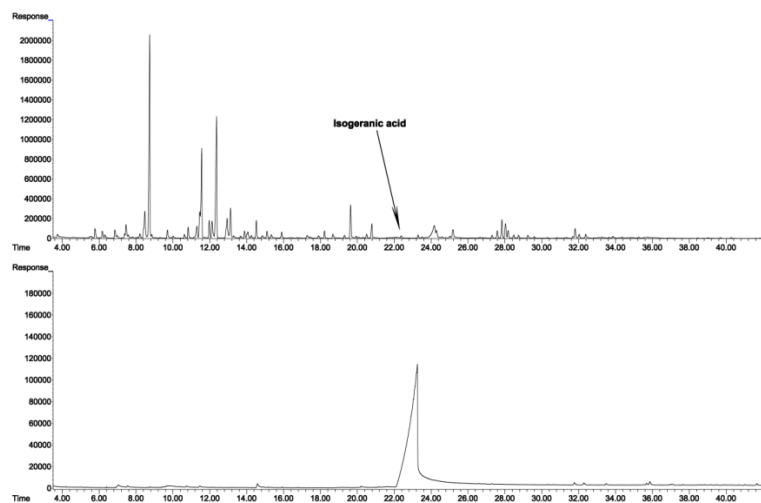


Figure S1. GC/FID chromatogram of the essential oil (up) and pure isogeranic acid (down)

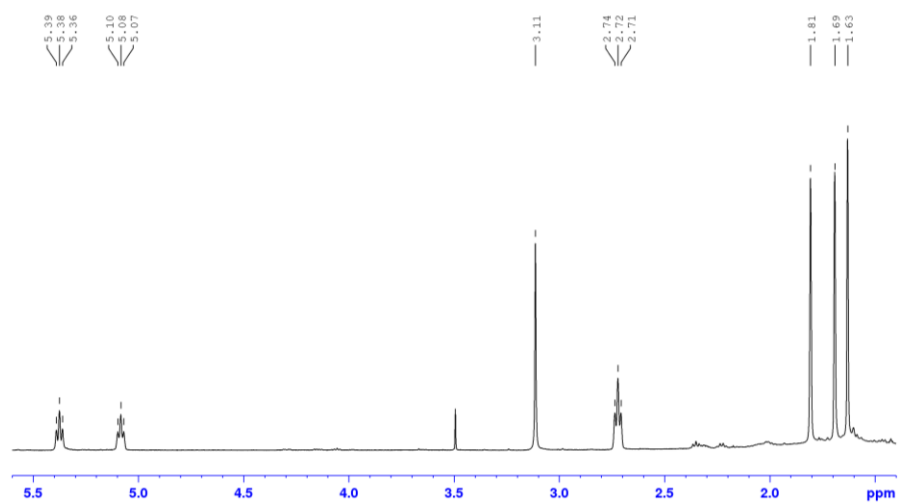


Figure S2. ^1H NMR spectrum of isogeranic acid

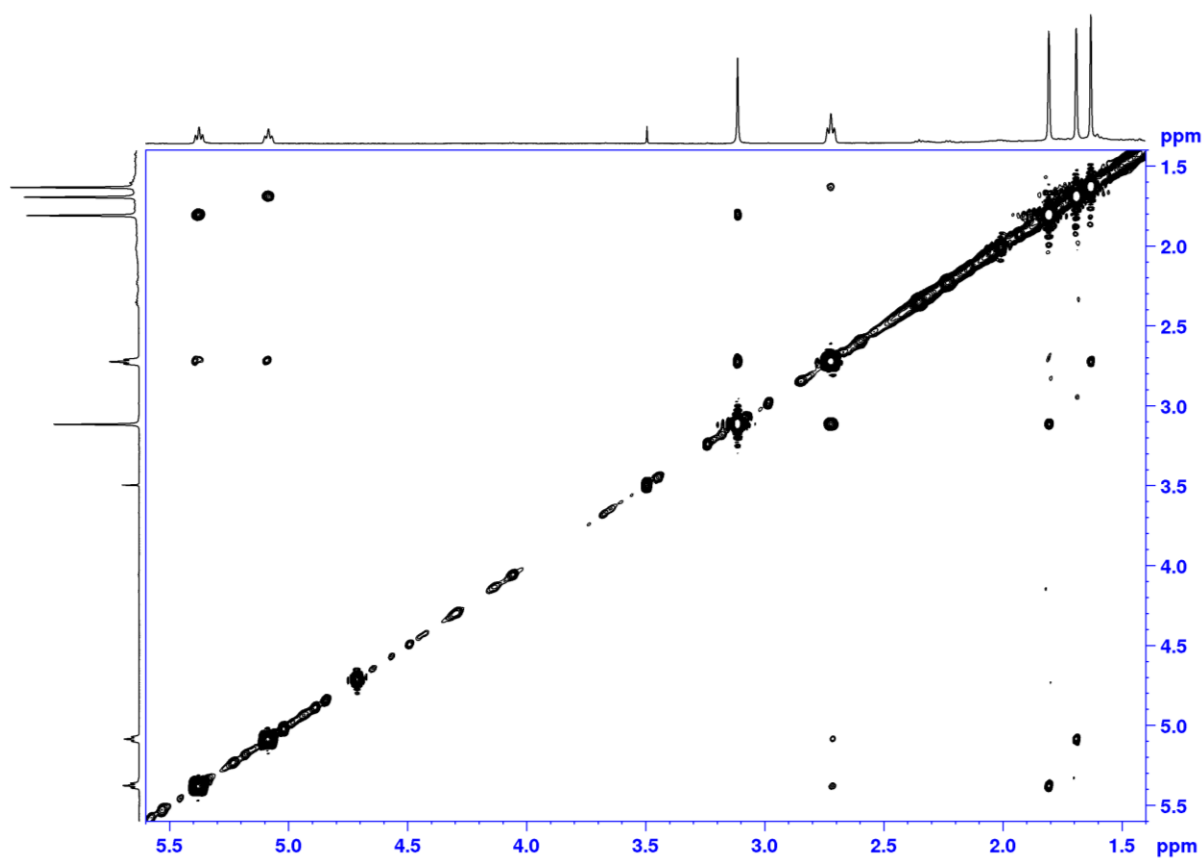


Figure S3. NOESY spectrum of isogeranic acid

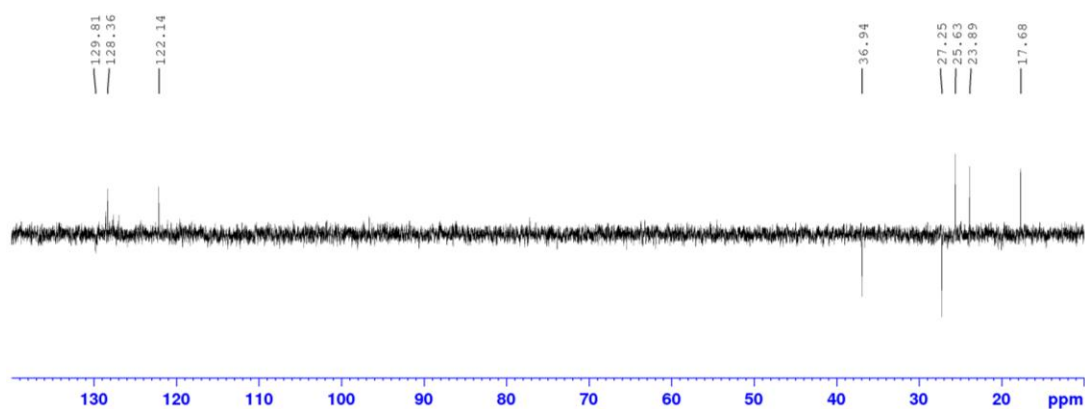


Figure S4. DEPT spectrum of isogeranic acid

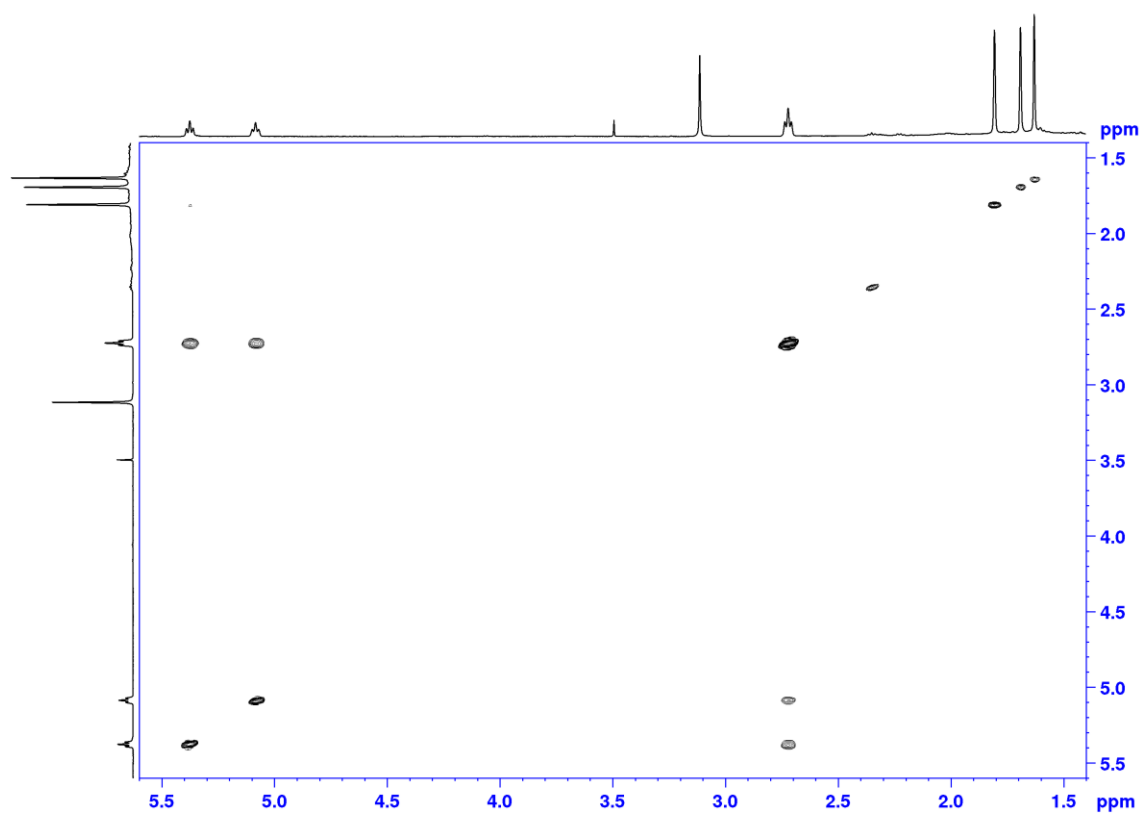


Figure S5. COSY spectrum of isogeranic acid

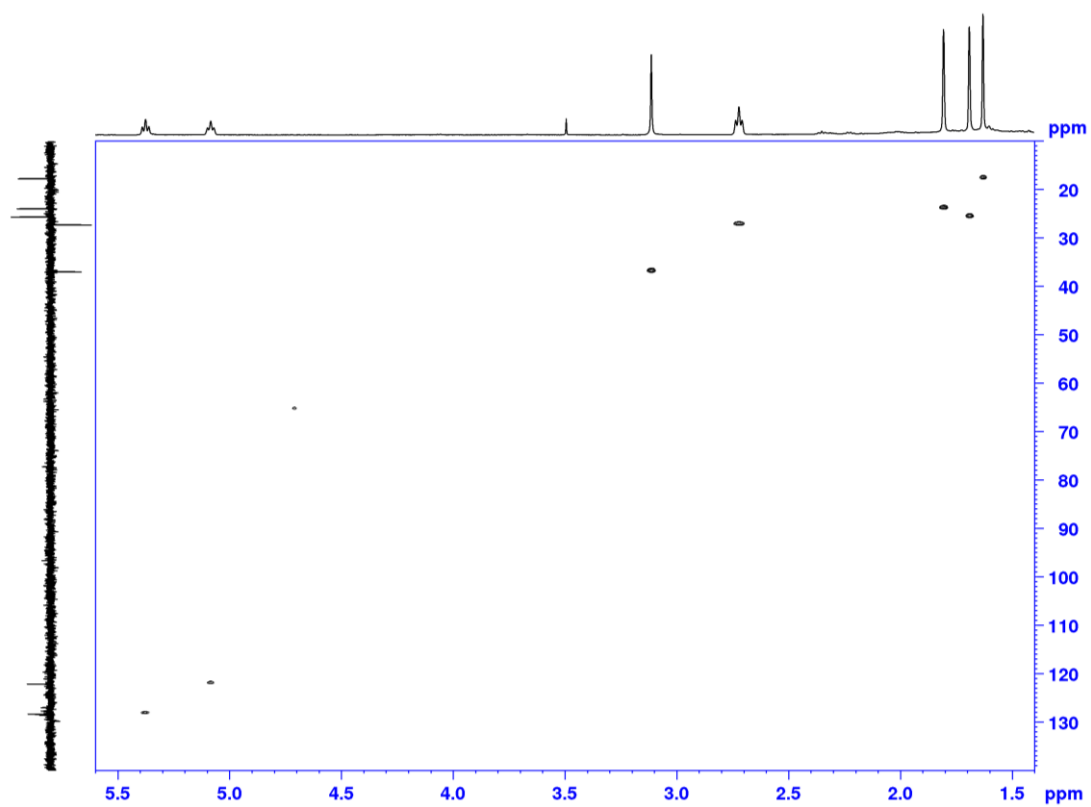


Figure S6. HSQC spectrum of isogeranic acid

42

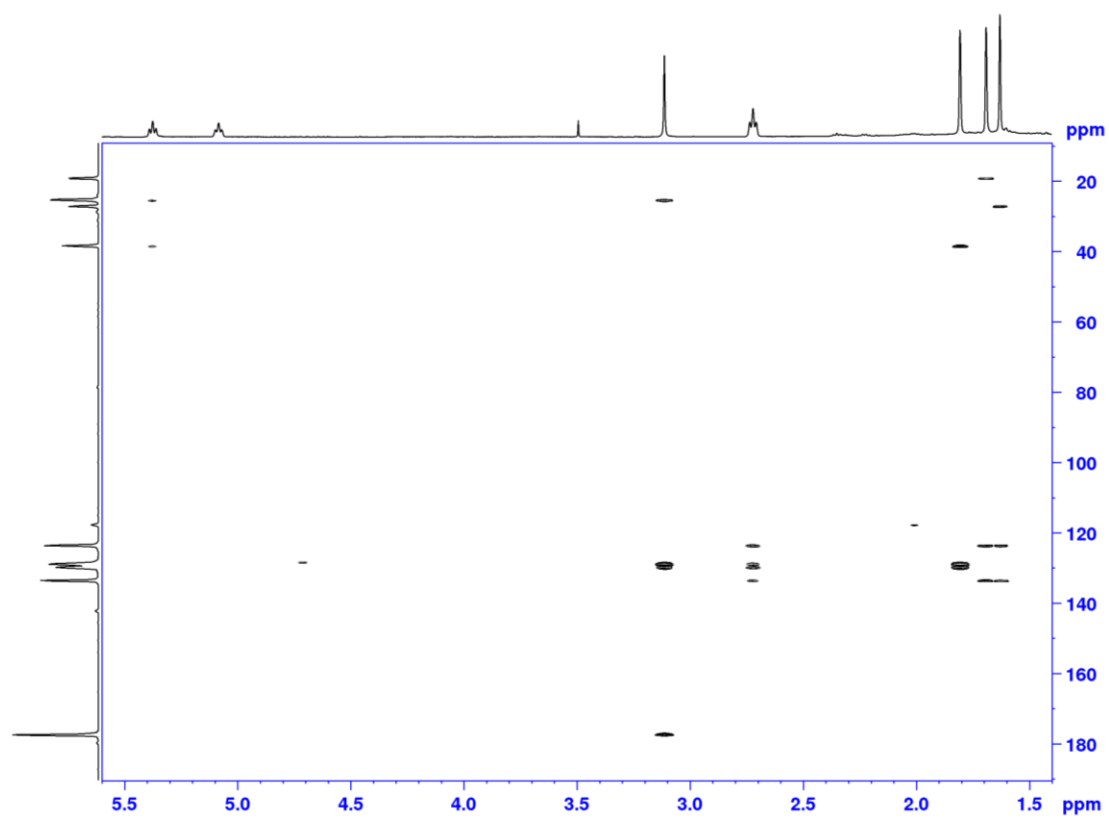


Figure S7. HMBC spectrum of isogeranic acid

43

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Table S1. Composition^a of the Essential Oil from *A. santonicum*

No.	Compound	RI ^b	Rt ^c (min)	% ^a
1	1,2,5,5-Tetramethyl-1,3-cyclopentadiene	817	3.741	0.4
2	NI	868	4.599	0.1
3	Tricyclene	906	5.489	0.2
4	α -Thujene	914	5.579	0.2
5	α -Pinene	921	5.771	1.1
6	Camphene	936	6.171	0.8
7	Thuja-2,4(10)-diene	942	6.315	0.4
8	Sabinene	966	6.852	0.8
9	β -Pinene	971	6.972	0.3
10	1,8-Dehydro cineole	985	7.379	0.3
11	Mesitylene	991	7.457	1.3
12	Yomogi alcohol	996	7.58	0.4
13	α -Phellandrene	999	7.808	0.2
14	NI	1006	8.081	0.1
15	α -Terpinene	1010	8.206	0.5
16	1,2,4-Trimethylbenzene	1015	8.425	0.6
17	<i>p</i> -Cymene	1016	8.473	2.8
18	1,8-Cineole	1022	8.739	18.8
19	Santolina alcohol	1026	8.848	0.3
21	NI	1036	9.300	0.1
22	γ -Terpinene	1046	9.698	0.9
23	<i>cis</i> -Sabinene hydrate	1056	10.006	0.3
24	Artemisia alcohol	1070	10.623	0.4
25	NI	1074	10.821	0.9
26	NI	1082	11.137	0.1
27	Linalool	1091	11.298	1.6
28	Filifolone	1094	11.455	2.6
29	<i>cis</i> -Thujone	1097	11.559	8.4
30	<i>trans</i> -Thujone	1110	11.969	1.6
31	Isophorone	1111	12.123	2.0
32	Chrysanthenone	1117	12.367	13.3
33	<i>trans</i> -Sabinol	1131	12.938	2.9
34	Camphor	1136	13.124	3.0
35	<i>p</i> -Mentha-1,7-dien-8-ol	1140	13.29	0.4
36	NI	1147	13.583	0.1
37	Sabina ketone	1150	13.669	0.2
38	Pinocarvone	1155	13.881	0.6
39	Borneol	1160	14.014	0.2

40	<i>p</i> -Mentha-1,5-dien-8-ol	1155	14.063	0.5
41	Santolinylnyl acetate	1159	14.253	0.2
42	Terpinen-4-ol	1171	14.522	1.5
43	Thuj-3-en-10-al	1178	14.848	0.4
44	α -Terpineol	1185	15.098	0.7
45	Myrtenal	1191	15.338	0.4
46	Verbenone	2004	15.898	0.8
47	Cumin aldehyde	1237	17.278	0.4
48	Carvone	1240	17.441	0.2
49	Piperitone	1250	17.891	0.3
50	<i>cis</i> -Chrysanthenyl acetate	1258	18.215	0.7
51	<i>p</i> -Mentha-1,8-dien-3-one	1268	18.673	0.5
52	NI	1276	19.013	0.1
53	Bornyl acetate	1277	19.303	0.3
54	<i>trans</i> -Sabinylnyl acetate	1285	19.636	3.3
55	Terpinen-4-ol acetate	1296	19.940	0.2
56	Carvacrol	1300	20.076	0.2
57	NI	1305	20.307	0.1
58	NI	1310	20.505	0.4
59	Filifolide A	1316	20.783	1.4
60	NI	1319	20.935	0.1
61	NI	1334	21.607	0.1
62	Piperitenone	1338	21.759	0.1
63	Isogeranic acid	1343	21.993	0.2
64	α -Terpinyl acetate	1345	22.127	0.1
65	NI	1348	22.205	0.1
66	NI	1352	22.371	0.2
67	Eugenol	1359	22.504	Tr
68	α -Copaene	1368	23.296	0.3
69	NI	1373	23.51	0.1
70	β -Bourbonene	1381	23.689	0.1
71	NI	1391	24.172	3.3
72	(<i>Z</i>)-Jasmone	1394	24.293	1.0
73	NI	1401	24.587	0.1
74	NI	1411	25.028	0.2
75	(<i>E</i>)-Caryophyllene	1415	25.184	1.2
76	α -Humulene	1449	26.641	0.1
77	(<i>E</i>)- β -Farnesene	1453	26.803	Tr
78	Dehydro sesquicineole	1464	27.303	0.3
79	NI	1471	27.586	0.6
80	Germacrene D	1479	27.837	1.8

81	β -Selinene	1482	28.033	1.6
82	Chrysanthenyl pentanoate	1486	28.174	0.8
83	Bicyclogermacrene	1494	28.48	0.4
84	NI	1501	28.742	0.3
85	NI	1512	29.247	0.3
86	δ -Cadinene	1516	29.597	0.1
87	NI	1534	30.304	0.1
88	NI	1550	30.818	0.1
90	NI	1562	31.359	0.1
91	NI	1571	31.691	0.2
92	Spathulenol	1574	31.81	1.1
93	Caryophyllene oxide	1580	32.023	0.5
94	NI	1587	32.382	0.6
95	Ledol	1599	32.842	0.1
96	NI	1604	33.073	0.1
97	NI	1607	33.172	0.1
98	NI	1609	33.282	0.1
99	NI	1619	33.636	0.1
100	NI	1624	33.855	0.2
101	γ -Eudesmol	1627	33.952	0.1
102	NI	1634	34.183	0.1
103	<i>epi</i> - α -Murrolol	1638	34.359	0.1
104	α -Cadinol andNI	1651	34.824	0.1
105	NI	1655	34.962	0.1
106	NI	1662	35.251	0.1
107	2,4-Dimethylether-phloroacetophenone	1671	35.552	0.1
108	NI	1672	35.633	0.1
109	Mustakone	1675	35.748	0.1
110	NI	1677	35.883	0.1
111	NI	1764	39.008	0.1
112	NI	1782	39.684	0.1
113	NI	1798	40.263	0.1

Monoterpene hydrocarbons	8.2
Oxygenated monoterpenes	67.5
Sesquiterpene hydrocarbons	5.6
Oxygenated sesquiterpenes	3.3
Normonoterpenes	2.6
Homomonoterpenes	1.0
Aromatics	1.9
Total	90.1

^aRelative percentages obtained by FID peak area normalization;^bRI: Non-isothermal Kovats retention indices on HP-5 MS (from temperature programming, using definition of Van den Dool and Kratz);^cRt: Retention time; tr: Trace (compound present in an amount less than 0.1 %);NI: Not identified.

Table S2. NMR Spectroscopic Data of Isogeranic Acid in CDCl₃

Atom No	¹ H, δ (mult, J_{in} Hz), integral	¹³ C, δ	NOESY	HMBC
1	1.69 (s), 3H	25.6	H-3	C-10
2	/	133.7	/	H-10, H-1, H-4
3	5.08 (t, 7.1), 1H	122.1	H-1, H-4	C-10, C-4, C-1
4	2.72 (t, 7.1), 2H	27.2	H-10, H-7, H-5, H-3	C-3, C-5, C-6, C-2
5	5.37 (t, 7.2), 1H	128.3	H-9, H-4	C-9, C-4, C-7
6	/	129.8	/	H-9, H-4
7	3.11 (s), 2H	36.9	H-9, H-4	C-5, C-6
8	/	177.6	/	H-7
9	1.80 (s), 3H	23.9	H-7, H-5	C-7
10	1.63 (s), 3H	17.7	H-4	C-4

Table S3. Minimum Inhibitory (MIC) and Bactericidal Concentration (MBC) of *A. santonicum* Essential Oil and Isogeranic Acid

Bacteria	Essential oil MIC MBC mg/mL	Isogeranic acid MIC MBC mg/mL	Streptomycin MIC MBC mg/mL	Ampicillin MIC MBC mg/mL
<i>Staphylococcus aureus</i>	15±2 50±3	/	0.04±0.003 0.10±0.006	0.25±0.008 0.40±0.009
<i>Bacillus cereus</i>	2±0.1 4±0.2	/	0.10±0.005 0.20±0.006	0.25±0.006 0.40±0.008
<i>Micrococcus flavus</i>	25±4 50±5	0.05 0.10	0.20±0.006 0.30±0.008	0.25±0.005 0.40±0.005
<i>Listeria monocytogenes</i>	15±6 50±3	0.05 0.10	0.20±0.006 0.30±0.005	0.40±0.005 0.50±0.006
<i>Pseudomonas aeruginosa</i>	4±0.3 6±0.3	0.075 0.10	0.20±0.003 0.30±0.002	0.75±0.008 1.25±0.040
<i>Salmonella typhimurium</i>	25±4 50±6	/	0.15±0.004 0.30±0.006	0.40±0.003 0.75±0.003
<i>Escherichia coli</i>	6±0.5 12±0.4	0.05 0.10	0.20±0.004 0.30±0.006	0.25±0.002 0.50±0.030
<i>Enterobacter cloacae</i>	6±0.4 12±0.6	/	0.25±0.006 0.50±0.008	0.40±0.003 0.75±0.020

/ - not tested

Table S4. Effects of Essential Oil and Isogeranic Acid on Inhibition of Biofilm Formation of *P.aeruginosa* (PAO1)

Samples	Inhibition of biofilm formation*		
	0.5MIC (%±SE)	0.25MIC (%±SE)	0.125MIC (%±SE)
Essential oil	-	1.52±0.62	42.54±2.77
Isogeranic acid	46.77±0.82	34.82±0.32	18.41±0.19
Ampicillin	65.28±0.65	58.94±0.46	85.24±0.37
Streptomycin	52.46±0.46	74.57±0.36	89.31±0.43

*Inhibition of biofilm formation values were calculated as: (mean A₆₂₀ control well -mean A₆₂₀ treated well)/(mean A₆₂₀ control well) ×100. Values are expressed as means ± SE.

- not activity

Table S5. Effects of *Artemisia santonicum* Essential Oil and Isogeranic Acid on Twitching and Protrusions Motility of *Pseudomonas aeruginosa* PAO1.

Agents	Colony diameter (mm ± SD)	Protrusions diameter (µm)	Colony Colour	Colony edge on microscope
Essential oil	11.00±2.65	-	white	reduced protrusion
Ampicillin	8.33±1.53	-	white	reduced protrusion

Streptomycin	6.00±1.73	-	white	reduced protrusion
Control (PAO1)	29.34±3.05	96-160	green	regular protrusion
Isogeranic acid	10.67±1.15	-	white	reduced protrusion

69

70 **Table S6.** Reduction of the Pyocyanin Production

Isogeranic acid	62.8	0.5
<i>A. santonicum</i> essential oil	60.6	1.9
Streptomycin	23.8	3.3
Ampicillin	32.5	1.5
Control (PAO1)	79.3	2.3

71