

Supplementary materials

The antimicrobial and antibiofilm *in vitro* activity of liquid and vapour phases of selected Essential Oils against *Staphylococcus aureus*

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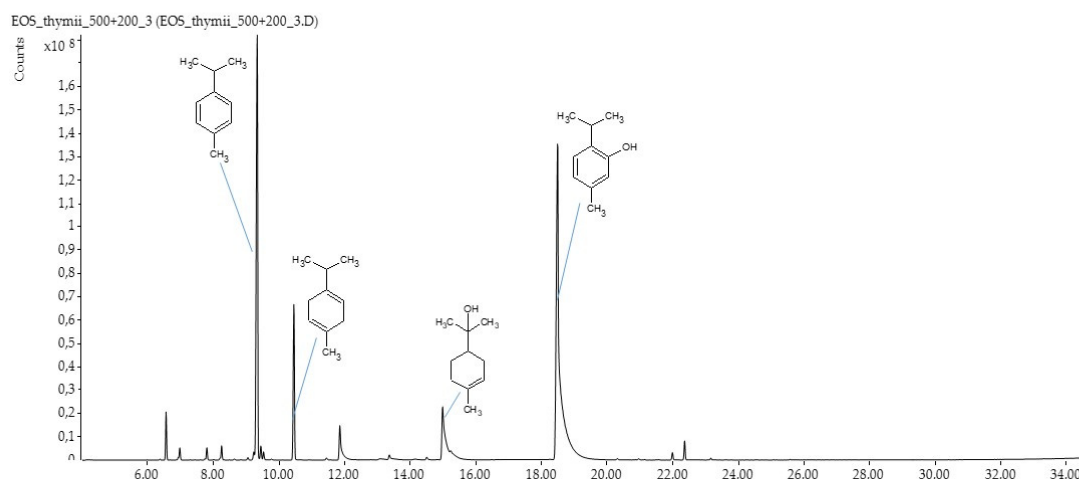
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Table S1. Ingredients of tested EOs measured with GC-MS (Gas Chromatography Mass Spectrometry). Components which are in line with Polish Pharmacopea XI standards are marked green colour, the ones which are not in line are marked red. **RI** - retention index, **RT** - retention time

A. Thyme oil



RI	RT	Compound	<i>T. vulgaris</i>
937	6.56	α -Pinene	2.20 \pm 0.09
949	6.98	Camphene	0.73 \pm 0.04
975	7.80	Sabinene	0.64 \pm 0.03
979	8.25	β -Pinene	0.81 \pm 0.05
1025	9.31	p-Cymene	26.91 \pm 0.99
1028	9.45	Limonene	0.77 \pm 0.04
1060	10.47	γ -Terpinene	8.60 \pm 0.03
1096	11.85	Linalool	3.45 \pm 0.15
1141	13.36	Camphor	0.66 \pm 0.06
1189	14.99	α -Terpineol	7.84 \pm 0.30
1289	18.49	Thymol	44.00 \pm 0.46
1419	22.36	β -Caryophyllene	1.00 \pm 0.05

Polish Pharmacopea XI ranges:

α - thujene: 0.2 per cent to 1.5 per cent

β -myrcene: 1.0 per cent to 3.0 per cent

α - terpinene: 0.9 per cent to 2.6 per cent

p- cymene: 14.0 per cent to 28.0 per cent

γ -terpinene: 4.0 per cent to 12.0 per cent

linalool: 1.5 per cent to 6.5 per cent

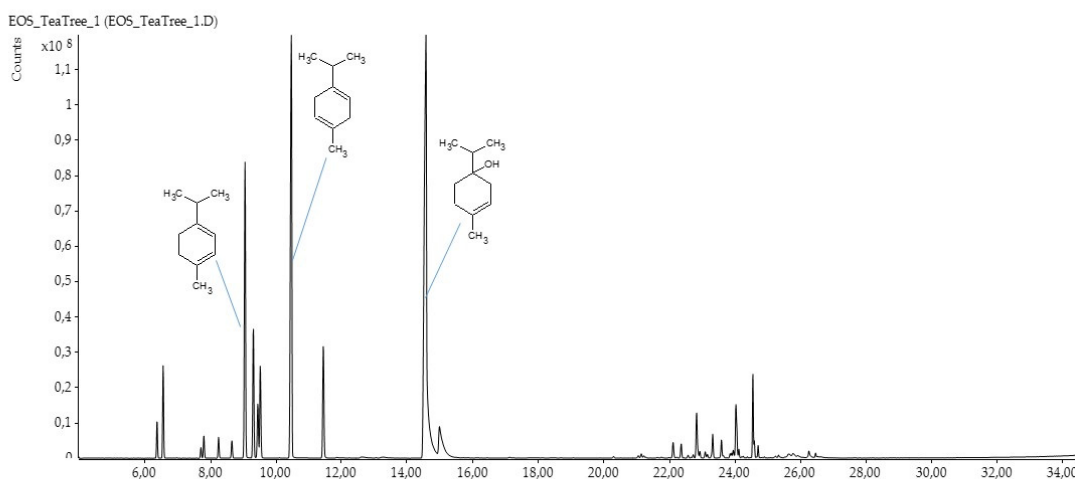
terpinen-4-ol: 0.1 per cent to 2.5 per cent

carvacrol methyl ether: 0.05 per cent to 1.5 per cent

thymol: 37.0 per cent to 55.0 per cent

carvacrol: 0.5 per cent to 5.5 per cent

B. Tea tree oil



RI	RT	Compound	<i>M. alternifolia</i>
929	6.37	α -Thujene	1.11 ± 0.02
937	6.56	α -Pinene	2.85 ± 0.06
975	7.80	Sabinene	0.75 ± 0.02
979	8.25	β -Pinene	0.69 ± 0.01
1005	8.65	α -Phellandrene	0.61 ± 0.01
1017	9.06	α-Terpinene	11.07 ± 0.17
1025	9.31	p-Cymene	4.69 ± 0.07
1028	9.45	Limonene	2.08 ± 0.05
1031	9.52	1,8-Cineole	3.34 ± 0.06
1060	10.47	γ-Terpinene	19.07 ± 0.27
1088	11.44	α -Terpinolene	4.34 ± 0.06
1177	14.58	Terpinen-4-ol	33.27 ± 0.79
1189	14.99	α -Terpineol	3.26 ± 0.13
1419	22.36	β -Caryophyllene	0.53 ± 0.01
1440	22.83	Aromadendrene	1.83 ± 0.03
1460	23.32	Alloaromadendrene	0.82 ± 0.02
1496	24.03	Viridiflorene	2.35 ± 0.04
1518	24.55	β -Cadinene	2.78 ± 0.03

Polish Pharmacopea XI ranges:

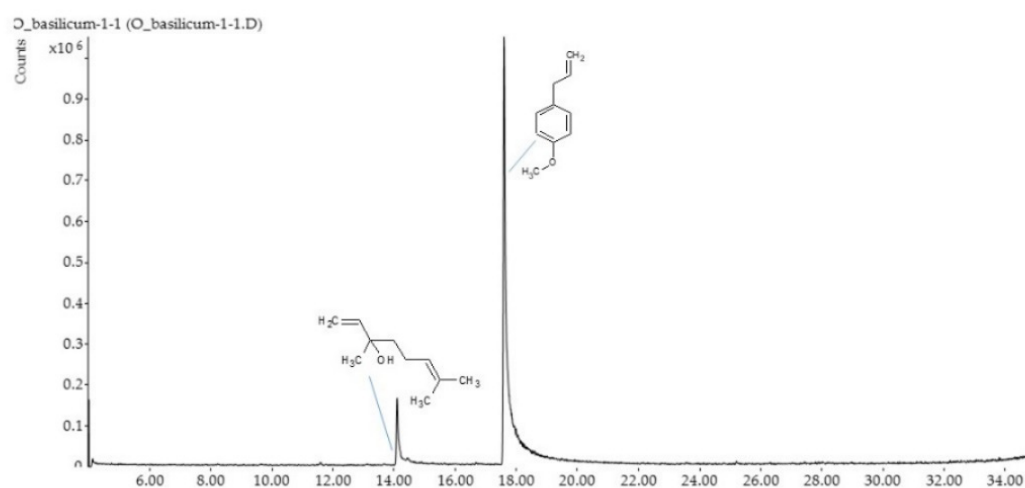
α -pinene: 1.0 per cent to 6.0 per cent

sabinene: maximum 3.5 per cent

α -terpinene: 5.0 per cent to 13.0 per cent

limonene: 0.5 per cent to 4.0 per cent
 cineole: maximum 15.0 per cent
 γ -terpinene: 10.0 per cent to 28.0 per cent
 p- cymene: 0.5 per cent to 12.0 per cent
 terpinolene: 1.5 per cent to 5.0 per cent
 terpinen-4-ol: minimum 30.0 per cent
 aromadendrene: maximum 7.0 per cent
 α - terpineol: 1.5 per cent to 8.0 per cent

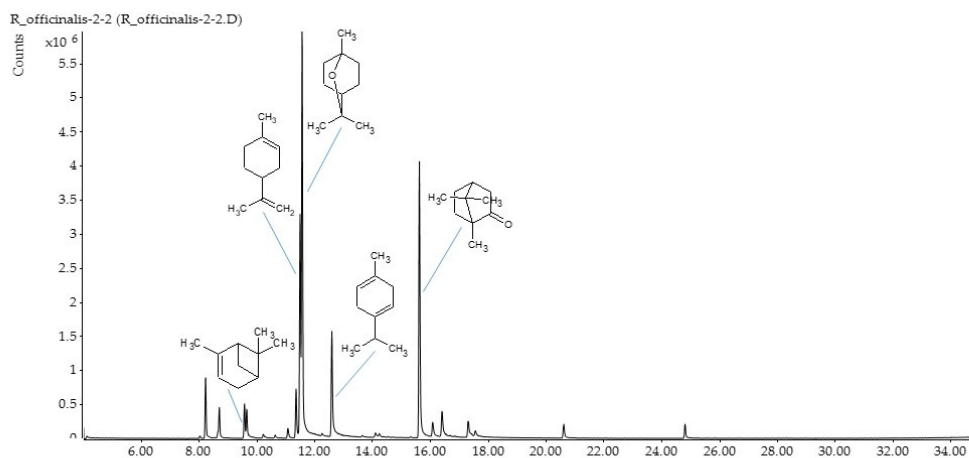
C. Basil oil



RI	RT	Compound	<i>O.basilicum</i>
1095	14.08	Linalool	10.69±1.13
1192	17.59	Methyl chavicol [Estragole]	89.31±1.13

Not included in Polish Pharmacopea XI

D. Rosemary oil



RI	RT	Compound	<i>R.officinalis</i>
896	8.22	Cyclofenchene	4.99±0.48
908	8.69	2-Bornene	3.00±0.29
924	9.57	β-Thujene	2.33±0.11
932	9.65	α-Pinene	2.58±0.28
988	10.22	Myrcene	0.38±0.14
1002	10.63	α-Phellandrene	0.23±0.07
1014	11.07	α-Terpinene	0.80±0.04
1022	11.35	o-Cymene	3.15±1.45
1024	11.49	Limonene	14.26±0.99
1026	11.57	1,8-Cineole [Eucalyptol]	30.12±1.74
1054	12.59	γ-Terpinene	8.21±0.35
1141	15.64	Camphor	21.97±0.77
1156	16.08	Isoborneol	1.53±0.06
1165	16.40	Borneol	2.69±0.09
1186	17.32	α-Terpineol	1.56±0.39
1284	20.61	Bornyl acetate	1.17±0.09
1417	24.80	Caryophyllene	0.85±0.21

Polish Pharmacopea XI ranges:

For rosemary oil, **Spanish type**, the percentages are within the following ranges:

α-pinene: 18 per cent to 26 per cent
 camphene: 8.0 per cent to 12.0 per cent
 β-pinene: 2.0 per cent to 6.0 per cent
 β-myrcene: 1.5 per cent to 5.0 per cent
 limonene: 2.5 per cent to 5.0 per cent
 cineole: 16.0 per cent to 25.0 per cent
 p-cymene: 1.0 per cent to 2.2 per cent

camphor: 13.0 per cent to 21.0 per cent

bornyl acetate: 0.5 per cent to 2.5 per cent

α -terpineol: 1.0 per cent to 3.5 per cent

borneol: 2.0 per cent to 4.5 per cent

verbenone: 0.7 per cent to 2.5 per cent

For rosemary oil, **Moroccan and Tunisian type**, the percentages are within the following ranges:

α -pinene: 9.0 per cent to 14.0 per cent

camphene: 2.5 per cent to 6.0 per cent

β -pinene: 4.0 per cent to 9.0 per cent

β -myrcene: 1.0 per cent to 2.0 per cent

limonene: 1.5 per cent to 4.0 per cent

cineole: 38.0 per cent to 55.0 per cent

p-cymene: 0.8 per cent to 2.5 per cent

camphor: 5.0 per cent to 15.0 per cent

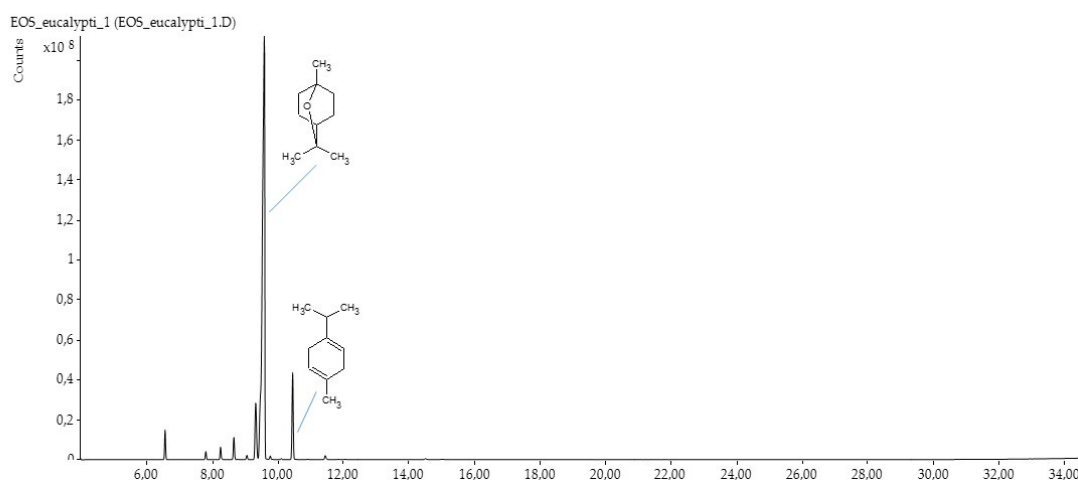
bornyl acetate: 0.1 per cent to 1.5 per cent

α -terpineol: 1.0 per cent to 2.6 per cent

borneol: 1.5 per cent to 5.0 per cent

verbenone: maximum 0.4 per cent

E. Eucalyptus oil

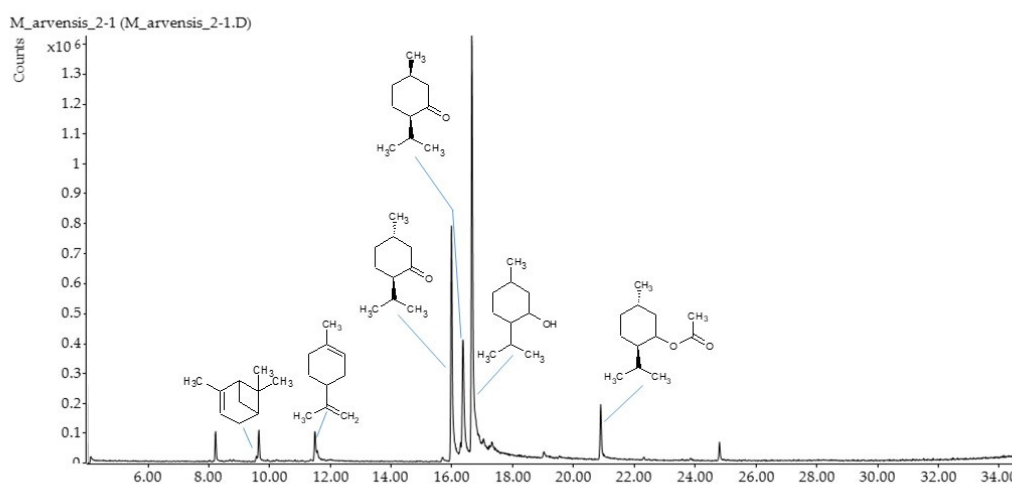


RI	RT	Compound	<i>E. globulus</i>
937	6.56	α -Pinene	2.30 ± 0.04
979	8.25	β -Pinene	1.09 ± 0.02
1005	8.65	α -Phellandrene	2.02 ± 0.02
1025	9.31	p-Cymene	6.89 ± 0.07
1031	9.52	1,8-Cineole	79.10 ± 0.61
1060	10.47	γ -Terpinene	8.16 ± 0.07

Polish Pharmacopea XI ranges

α -pinene: 0.05 per cent to 10.0 per cent
 β -pinene: 0.05 per cent to 1.5 per cent
sabinene: maximum 0.3 per cent
 α -phellandrene: 0.05 per cent to 1.5 per cent
limonene: 0.05 per cent to 15.0 per cent
1,8-cineole: minimum 70.0 per cent
camphor: maximum 0.1 per cent

F. Menthol mint oil

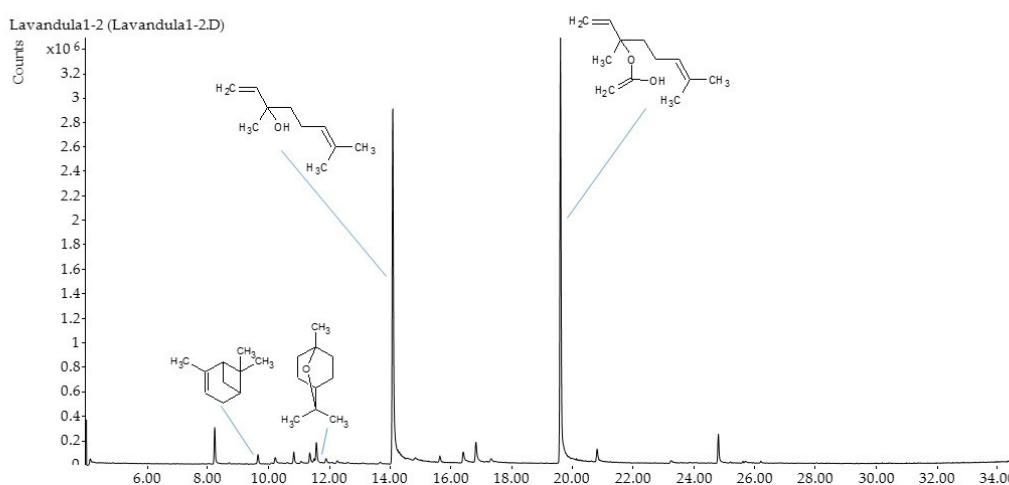


RI	RT	Compound	<i>M.arvensis</i>
896	8.22	Cyclofenchene	2.68±0.17
932	9.65	α -Pinene	3.01±0.17
1024	11.49	Limonene	3.78±0.27
1148	15.98	Menthone	24.53±0.23
1158	16.36	Isomenthone	13.54±1.75
1167	16.66	Menthol	45.57±2.21
1294	20.89	Menthyl acetate	5.61±0.36
1417	24.80	Caryophyllene	1.27±0.57

Polish Pharmacopea XI ranges:

limonene: 1.5 per cent to 7.0 per cent
 cineole: maximum 1.5 per cent
 menthone: 17.0 per cent to 35.0 per cent
 isomenthone: 5.0 per cent to 13.0 per cent
 menthyl acetate: 1.5 per cent to 7.0 per cent
 isopulegol: 1.0 per cent to 3.0 per cent
 menthol: 30.0 per cent to 50.0 per cent
 pulegone: maximum 2.5 per cent
 carvone: maximum 2.0 per cent
 The ratio of cineole content to limonene content is less than 1.

G. Lavender oil



RI	RT	Compound	<i>L. angustifolia</i>
896	8.22	Cyclofenchene	3.66±0.35
932	9.65	α -Pinene	0.94±0.04
988	10.22	Myrcene	0.86±0.13
1008	10.83	3-Carene	1.19±0.12
1022	11.35	o-Cymene	1.19±0.12
1026	11.57	1,8-Cineole [Eucalyptol]	2.56±0.39
1095	14.08	Linalool	37.76±1.18
1141	15.64	Camphor	0.84±0.10

1165	16.40	Borneol	2.04±0.56
1174	16.82	Terpinen-4-ol	3.03±0.46
1254	19.60	Linalyl acetate	41.13±0.40
1288	20.81	Lavandulyl acetate	1.80±0.22
1417	24.80	Caryophyllene	3.47±0.66

Polish Pharmacopea XI ranges

limonene: maximum 1.0 per cent
 1,8-cineole: maximum 2.5 per cent
 3-octanone: 0.1 per cent to 5.0 per cent
 camphor: maximum 1.2 per cent
 linalool: 20.0 per cent to 45.0 per cent
 linalyl acetate: 25.0 per cent to 47.0 per cent
 terpinen-4-ol: 0.1 per cent to 8.0 per cent
 lavandulyl acetate: minimum 0.2 per cent
 lavandulol: minimum 0.1 per cent
 α-terpineol: maximum 2.0 per cent

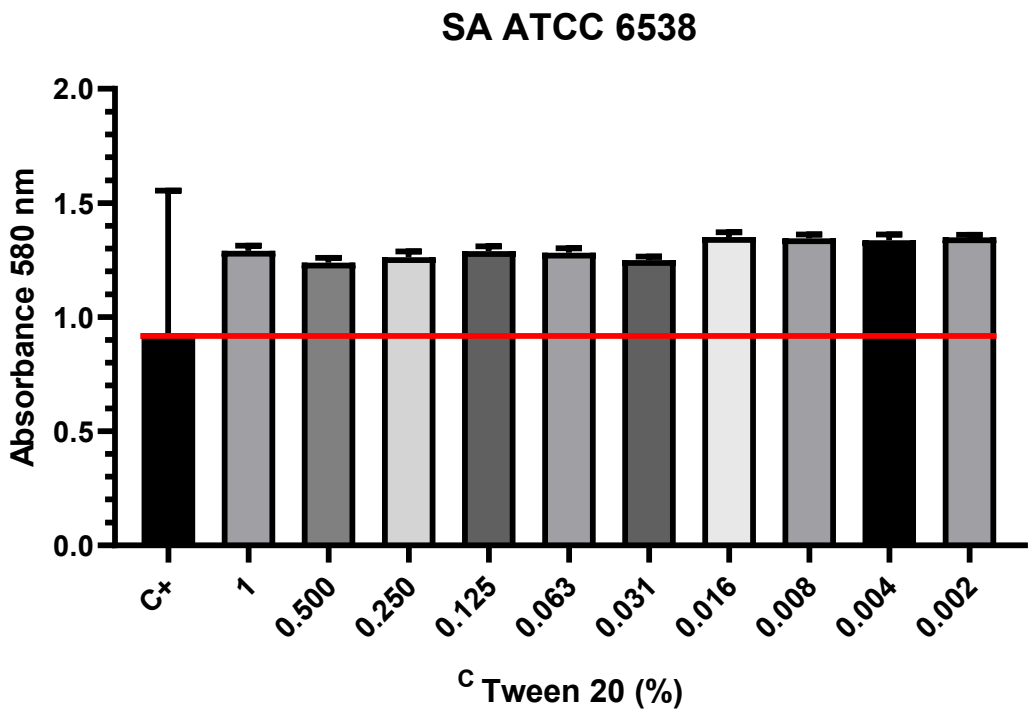


Figure S1. Influence of different concentrations of Tween 20 [(%) (v/v)] on planktonic forms of *S. aureus* ATCC 6538 strain. C+ untreated cells.

MBC-B (%)

T- EO		TT-EO		B-EO		R-EO		M-EO		L-EO	
Strain	MBC-B	Strain	MBC-B	Strain	MBC-B	Strain	MBC-B	Strain	MBC-B	Strain	MBC-B
SA 5	0.05	SA 2	25	SA 4	50	2	12.5	SA 32	50	SA 2	25
SA 6	0.1	SA 4	12.5	SA 29	50	4	6.3	SA 34	50		
SA 7	0.05	SA 5	25	SA 32	12.5	7	50	SA 35	50		
SA 10	0.05	SA 27	50	SA 34	25	27	12.5	SA ATCC 33591	25		
SA 26	0.05	SA 28	3.1	SA ATCC 33591	25	33	25	SA ATCC 6538	50		
SA 27	0.05	SA 32	6.3	SA ATCC 6538	12.5	34	25				
SA 32	0.1	SA 33	6.3			35	50				
SA 33	0.1	SA 34	6.3			SA ATCC 33591	12.5				
SA ATCC 6538	0.4	SA ATCC 33591	1.6								

Table S2. The MBC- B [(%) (v/v)] (minimal bactericidal concentration for biofilm) values of EOs emulsions. T-EO- thyme oil, TT-EO- tea tree oil, B- EO- basil oil, R-EO- rosemary oil, M-EO- menthol mint oil, L- EO- lavender oil