Evaluation of Antibacterial Effect of Odor Eliminating Compounds

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Summary

Antibiotic activity of ten commercially available odor eliminating compounds were tested on seven bacteria including odor causing *Staphylococcus hominis* and common pathogens *Pseudomonas aeruginosa* and Methicillin resistant *Staphylococcus aureus* (MRSA). The antibiotic activity of each compound was determined via inhibition zone assay, minimum inhibitory concentration, and time-dependent bacterial killing. Based on our results, Compound 3 (Silver B) had the most effective antibacterial activity followed closely by Compound 4 (Silver A). Other commercial compounds had varying degree of antibacterial activity on the tested bacteria. Trade and brand names used in this publication are given for information purposes only. No guarantee, endorsement, or discrimination among comparable products is intended or implied by the listed researchers or Auburn University.

Materials and Methods

Inhibition zone assay

Antibacterial activity of each compound (Table 1) against a panel of selected bacteria (Table 2) was determined using a modified inhibition zone assay. For every assay, each bacterium was freshly grown from a frozen stock. Briefly, a colony from a freshly streaked plate was inoculated into Lysogeny Broth (LB) medium, grown overnight at 37°C with shaking at ~220 rpm, subcultured the next day into fresh LB medium and grown at 37°C with shaking at ~220 rpm to early log-phase of growth (OD_{600} = 0.3 ± 0.05), and diluted to ~2.5×10^7 CFU/ml. The bacterial cells were evenly spread onto each petri plate using soft agar to obtain a uniform lawn. The antibacterial activity of each compound was examined by placing 7 sterilized filter paper disks (5 × 5 mm) uniformly on the bacterial lawn in each plate. The paper disks were treated with one

of the following samples on the same plate, respectively: 20 µl of the first five compounds, 1 µl of ampicillin (= 25 µg/disk), and 1 µl of Spor-Klenz® (Steris Corp) as the positive control. Another plate with 7 sterilized filter paper disks were treated with one of the following samples, respectively: 20 µl of the other five compounds (Compounds 6-10), and 1 µl of ampicillin (= 25 µg/disk), and 1 µl of Spor-Klenz® as the control. Every assay was repeated 6 times, each with 3 replicates, to acquire an N of 18 for each treatment.

Minimum inhibitory concentration (MIC) determination

The antibacterial activity of the selected compounds (Compounds 3, 4 and 7) was further studied by employing a microdilution method, using Mueller-Hinton (MH) broth in a 96-well flat-bottom microtiter plate. Each bacterium (*P. aeruginosa*, MRSA, *S. hominis*, and *S. epidermidis*) was grown overnight from a single colony in MH broth and diluted to OD_{600}=0.08. 1:1 serial dilutions of the aforementioned compounds were performed by adding the sterilized distilled water. In total, 5 dilutions were performed. 20 µl of each dilution were distributed in 96-well plates, as well as 100 µl of diluted bacterial suspension and a growth control (containing culture broth plus 20 µl of sterilized distilled water, without any odor removal compounds) of the four bacteria, respectively. The microtiter plates were incubated at 37ºC for 24 h with shaking in BioTek Synergy HT microtiter plate reader. Bacterial growth was monitored every 5 min as OD_{600} during the incubation. The experiment was repeated twice.

Killing Curve

To measure organisms' ability to survive in odor eliminating compounds (Compounds 3, 4, and 7), four bacteria (*P. aeruginosa*, MRSA, *S. hominis*, and *S. epidermidis*) grown overnight in LB at 37ºC with aeration were washed twice and diluted

to OD_{600} = 1.0 ± 0.1 in 1x Phosphate buffered saline (PBS) supplemented with 3x trace mineral, respectively. The time at which the cells were diluted was taken as time 0, and the compound was added into the diluted cells at the ratio of 1:5 and incubated for 24 h at room temperature. Viable cell counting was performed to determine colony forming units (CFU/ml) at times 0, 5 min, 15 min, 30 min, 1 h, 2 h, 4 h, 8 h, 12 h, and 24 h by serial dilutions. All plates were incubated in 37ºC for at least 12 h and colonies were counted to determine the viable cell counts.

Table 1. List of bacteria used in the study

Bacterium	Gram Stain
Staphylococcus aureus	+
Methicillin-resistant *Staphylococcus aureus* (MRSA)	+
Streptococcus pyogenes	+
Staphylococcus epidermidis	+
Staphylococcus hominis	+
Pseudomonas aeruginosa (PAO1)	-
Acinetobacter baumannii (AYE)	-

Table 2. List of compounds used in the study

Compound	Compound name
1	Outdoor field spray
2	J-lab odor eliminator
3	Silver product B
4	Silver product A
5	Evolve 3D
6	Scent killer
7	Scent A-Way Wax
8	Control freak
9	3 in 1 odor stop field spray
10	B-tech

Results

Susceptibility of various bacteria associated with either body odor or skin infections to odor-eliminating compounds was determined via three independent assays:

zone of inhibition, minimum inhibitory concentration (MIC), and time-dependent killing. The zone of inhibition is one of the most commonly used techniques to determine antibiotic effectiveness. We tested six odor-causing bacteria for their susceptibility to the listed compounds by measuring diameters of inhibitory zones on bacterial lawns. Compounds 3, 4 and 10 displayed broad inhibitory activity against all six bacteria tested (Table 3), while compounds 1, 5 and 6 showed the least antibacterial effect by producing zones of clearance only on one or two bacteria (*S. aureus*, *S. pyogenes*, *P. aeruginosa* or *A. baumannii*). Compounds 7 and 9 had inhibitory effect on five bacteria, but not against *S. hominis,* while compound 8 lacked antagonistic effect against *P. aeruginosa*.

The MIC determines the minimum effective concentration of a compound that prevents growth of a microorganism. We determined the MIC of Compounds 3, 4 and 7 against four selected bacteria: *P. aeruginosa*, MRSA, *S. hominis*, and *S. epidermidis*. We used the same amount of compound for each assay. The data suggest that compound 7 is less effective (Fig. 3A) in inhibiting the growth of *P. aeruginosa* when compared to Compounds 3 (Fig. 1A) and 4 (Fig. 2A). The most diluted concentration of Compound 3 and the moderate concentration of Compound 4 demonstrated inhibitory effect while only the undiluted concentration of Compound 7 displayed growth inhibition. For MRSA and *S. hominis*, similar pattern was observed for each compound (Fig. 1B-3B; Fig. 1C-3C). Interestingly, all of the diluted compounds showed inhibiting effect on the growth of *S. epidermidis*. However, the growth of *S. epidermidis* with the most diluted Compound 3 was lower than the same dilution of Compounds 4 and 7, respectively (Fig. 1D-3D).

Table 3. Antibacterial activity of odor eliminating compounds

Compound	Bacteria						
	P. aeruginosa	MRSA	*A. baumanii*	*S. aureus*	*S. epidermidis*	*S. pyogenes*	*S. hominis*
1	0	0	0	15.63±0.77	0	19.13±0.56	0
2	0	9.55±0.36	10.08±0.39	10.91±0.62	14.79±0.58	10.71±0.74	37.75±0.21
3	16.98±0.3	14.17±0.2	14.75±0.58	15.94±0.6	15.4±0.58	15.25±0.48	14.13±0.19
4	14.13±0.4	10.41±0.45	9.94±0.67	11.81±0.57	11.70±0.67	10.32±0.44	11.96±0.31
5	9.03±0.11	0	0	0	0	0	0
6	9.85±0.26	0	9.24±0.29	0	0	0	0
7	14.55±0.58	9.71±0.32	9.05±0.38	10.26±0.30	10.14±0.48	9.91±0.62	0
8	0	17.09.±0.53	11.75±0.54	17.33±0.45	21.85±0.59	16.41±0.18	19.32±0.28
9	10.12±0.36	15.08±0.43	14.94±0.59	14.13±0.49	12.72±0.43	10.57±0.38	0
10	13.39±0.42	21.25±0.49	15.14±0.42	20.73±0.54	29.97±0.66	25.86±0.42	28.3±0.30

Zone of clearing was measured in mm.

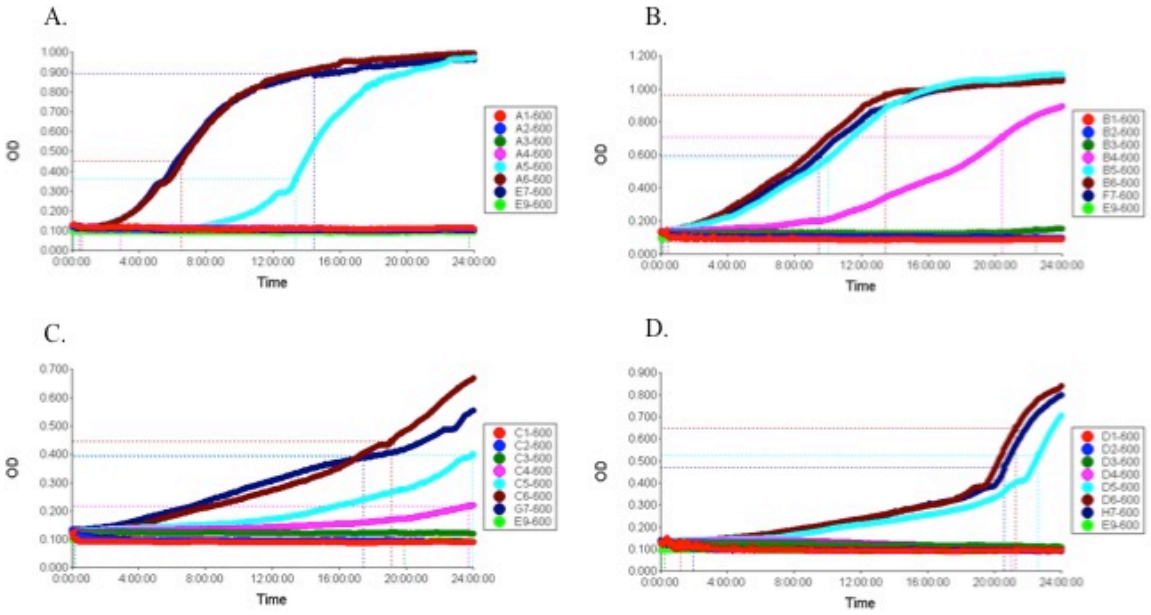


Fig. 1. MIC of Compound 3 on bacterial growth. (A) *P. aeruginosa* (B) MRSA (C) *S. hominis* and (D) *S. epidermidis*. A1, B1, C1 and D1 are the undiluted compound, A2-A6, B2-B6, C2-C6, D2-D6 followed 1x serial dilutions. E7, F7, G7, and H7 correspond to the positive control of bacterial growth curve.

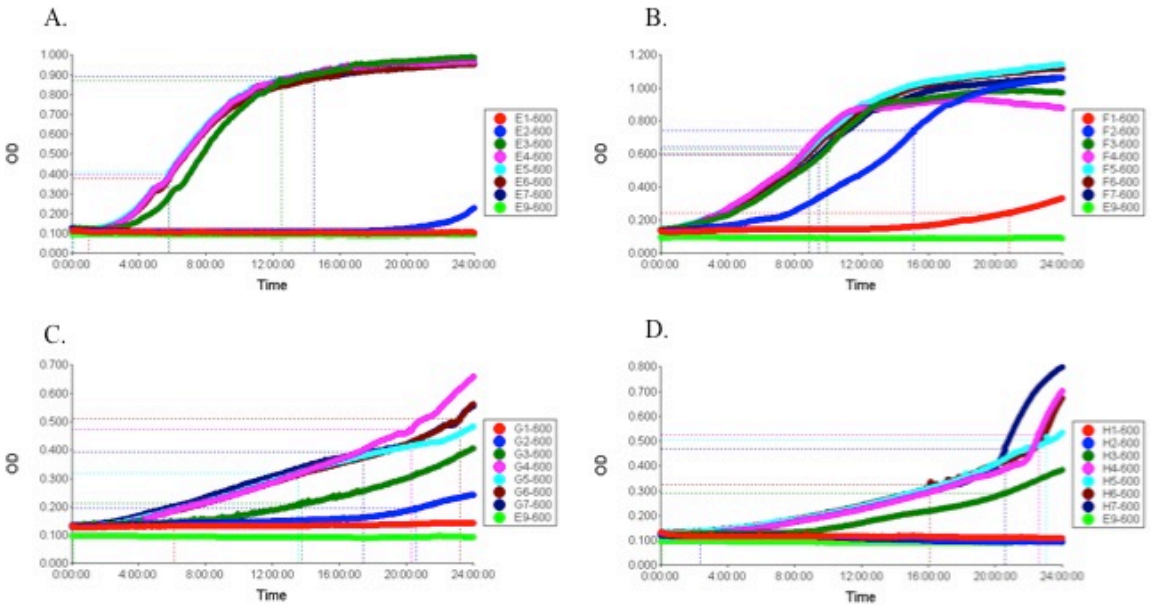


Fig. 2. MIC of Compound 4 on bacterial growth. (A) *P. aeruginosa* (B) MRSA (C) *S. hominis* and (D) *S. epidermidis*. E1, F1, G1 and H1 are the undiluted compound, E2-E6, F2-F6, G2-G6, H2-H6 followed 1x serial dilutions. E7, F7, G7, and H7 correspond to the positive control of bacterial growth curve.

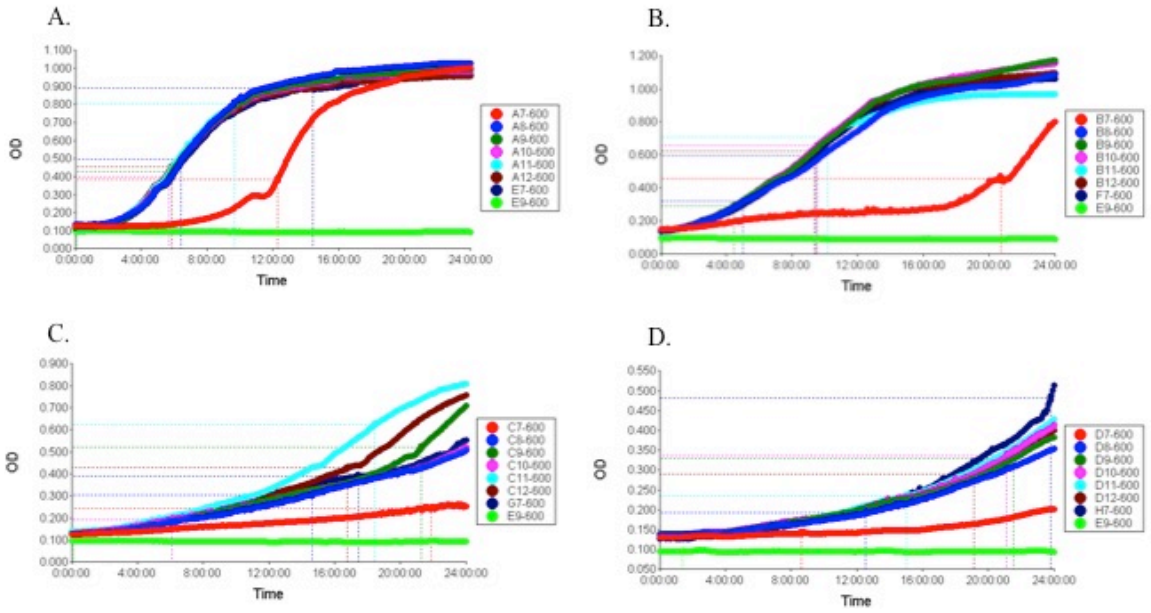


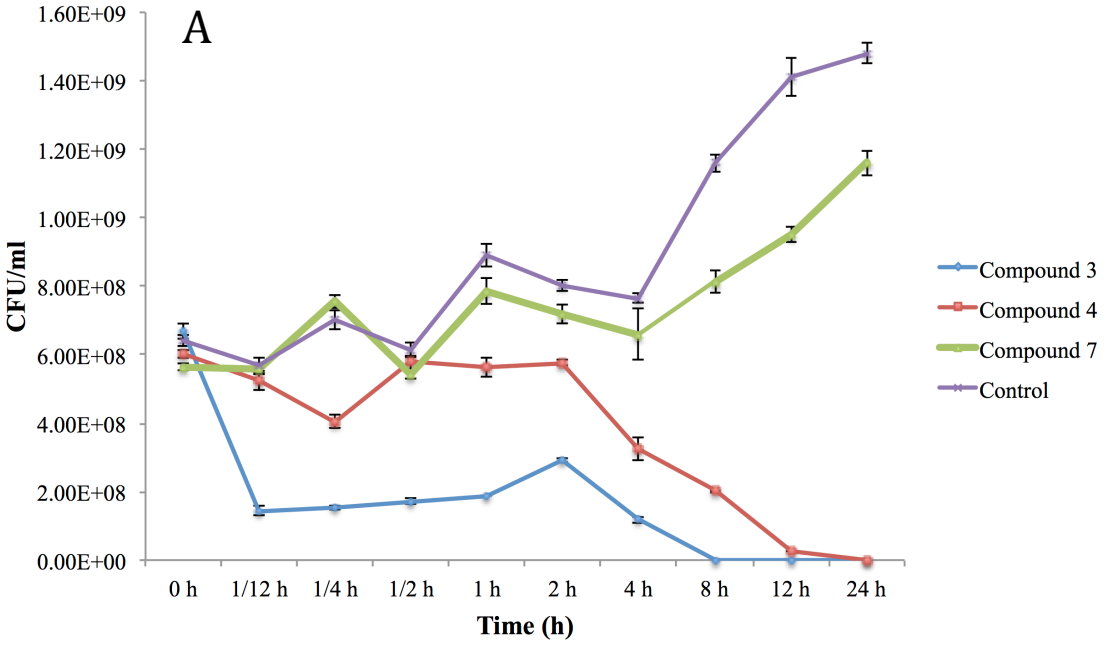
Fig. 3. MIC of Compound 7 on bacterial growth. (A) *P. aeruginosa* (B) MRSA (C) *S. hominis* and (D) *S. epidermidis*. A7, B7, C7 and D7 are the undiluted compound, A8-A12, B8-B12, C8-C12, D8-D12 followed 1x serial dilutions. E7, F7, G7, and H7 correspond to the positive control of bacterial growth curve.

Finally, we determined the bactericidal activity of three compounds against four bacteria. This assay measures the relative rate of bactericidal activity of each compound as a function of time. Figure 4 shows the actual measurement of CFU while Figure 5 shows the percentage of cells surviving the treatment with the respective compound. Tables 4 through 7 list the percentage of cells surviving the treatment as a function of time. The results demonstrate that Compound 3 has the most effective bactericidal activity with regards to both rate of killing and the final extent of killing. Within the first five minutes, Compound 3 killed 78% of *P. aeruginosa*, 93% of MRSA, 99.9% of *S. hominis*, and 69% of *S. epidermidis*. After 24 hours, Compound 3 killed 99.99% of *P. aeruginosa*, 99.9% of MRSA, 99.9999% of *S. hominis,* and 99.9999% of *S. epidermidis*. Growth of *P. aeruginosa* in PBS supplemented with trace minerals was not unexpected

because this bacterium is known to survive and grow even in distilled water. Compound 4 killed 57% of *P. aeruginosa*, 34% of MRSA, 57% of *S. hominis,* and 66% of *S. epidermidis* within the first five minutes. In 24 hours, Compound 4 killed 99.996% of *P. aeruginosa*, 60.4% of MRSA, 99.95% of *S. hominis*, and 99.99999% of *S. epidermidis*. In contrast to Compounds 3 and 4, Compound 7 killed 0% of *P. aeruginosa,* 59% of MRSA, 3% of *S. hominis*, and 37% of *S. epidermidis* within the first five minutes. In 24 hours, Compound 7 killed 0% of *P. aeruginosa*, 94.3% of MRSA, 56% of *S. hominis*, and 68% of *S. epidermidis*. The difference in antibiotic activity of Compound 7 on *P. aeruginosa* between the zone of inhibition assay and the time-dependent killing curve may be due to its effectiveness against actively growing bacterium (zone of inhibition) versus the organism with very slow metabolism (killing curve). Bacteria on clothes are likely to have sedentary metabolism.

Conclusion

In all three assays, Compound 3 (Silver B) was superior in its antibiotic activity than all other compounds. In addition, it was effective against all bacteria tested including the odor-causing bacterium *S. hominis.* Compound 4 (Silver A) was almost as effective. Based on our results, we conclude that Compound 3 is the most effective compound to eliminate either odor-causing bacteria or common skin-infection causing pathogens.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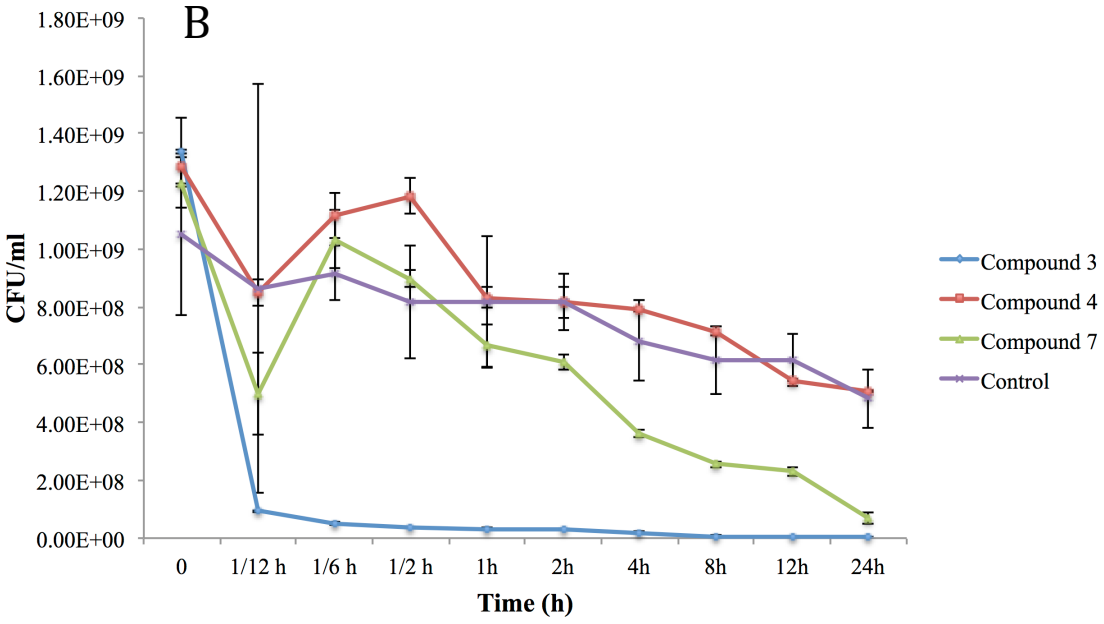
1.20E+09
1.00E+09
8.00E+08
6.00E+08
4.00E+08
2.00E+08
0.00E+00
CFU/ml
0 h
1/12 h
1/4 h
1/2 h
1 h
2 h
4 h
8 h
12 h
24 h
Time (h)
Compound 3
Compound 4
Compound 7
Control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1.00E+09
8.00E+08
6.00E+08
4.00E+08
2.00E+08
0.00E+00
CFU/ml
0
1/12 h
1/6 h
1/2 h
1h
2h
4h
8h
12h
24h
Time (h)
Compound 3
Compound 4
Compound 7
Control

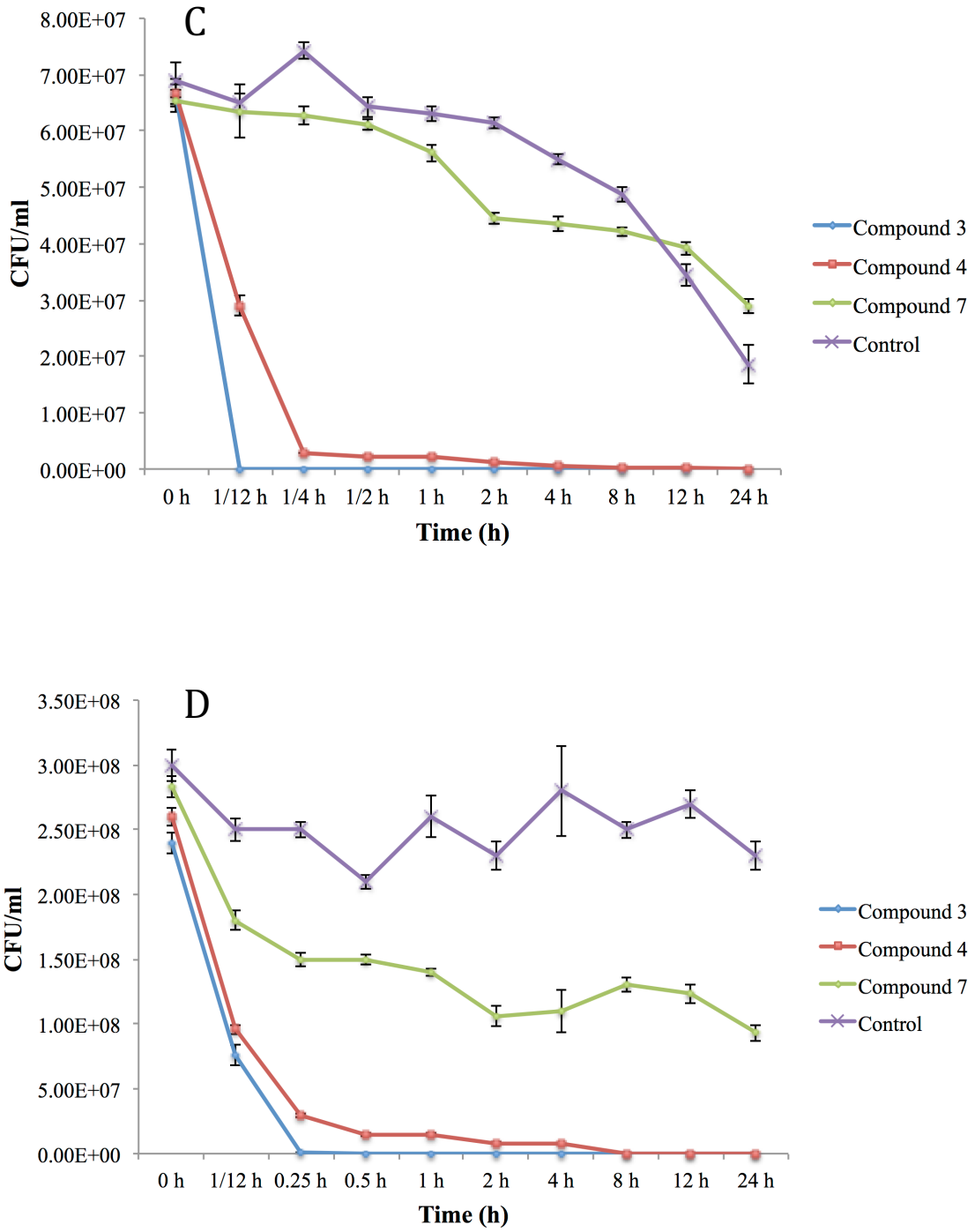
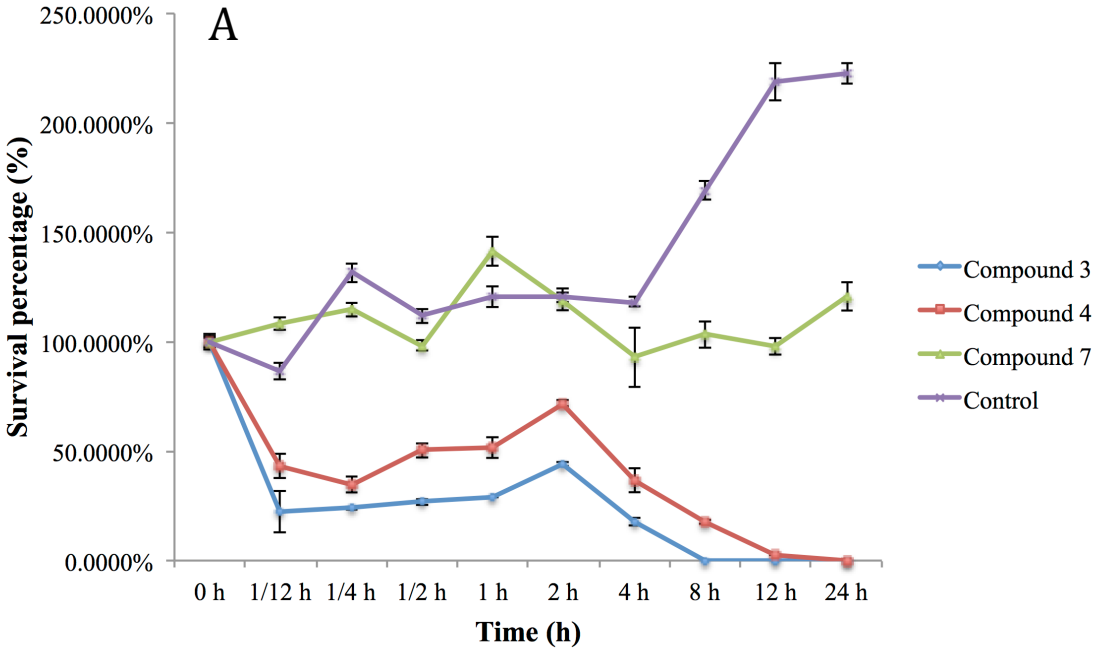
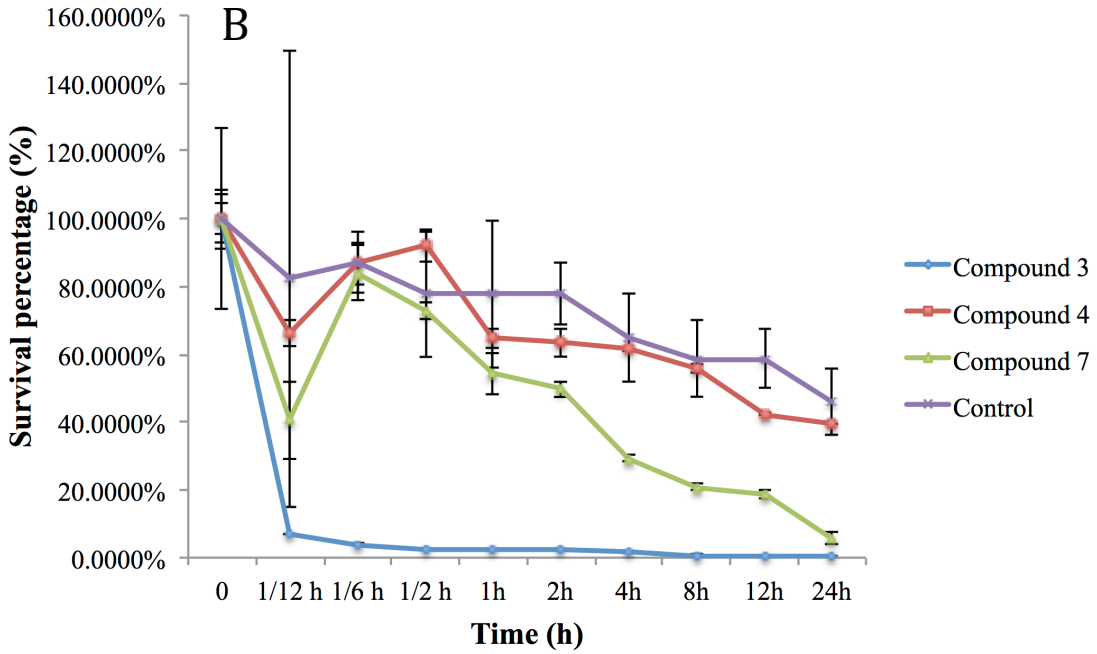
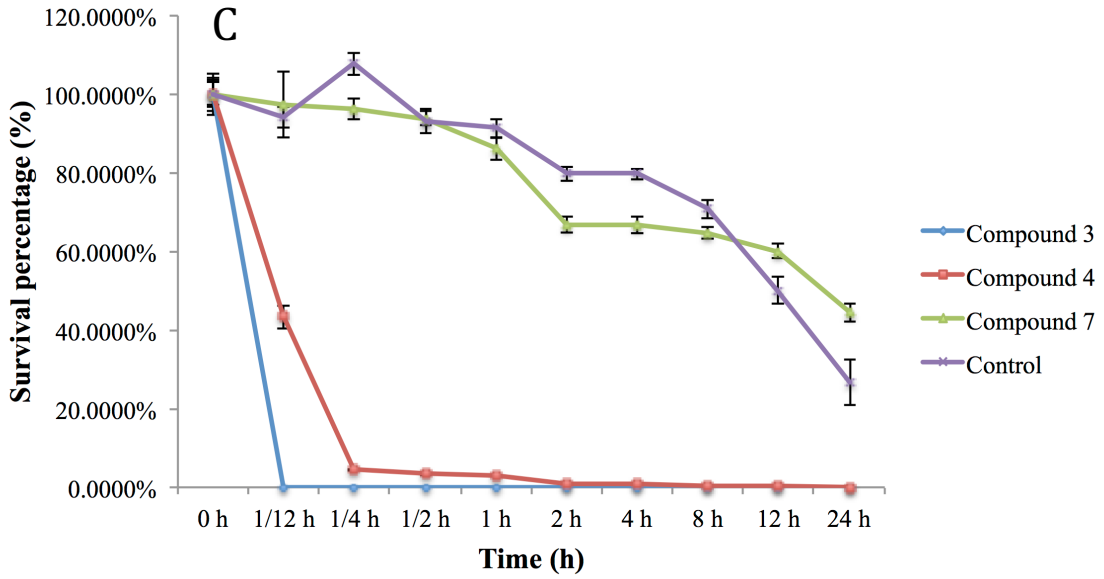


Fig. 4. Bactericidal activity of Compounds 3, 4, and 7 represented as CFU. (A) *P. aeruginosa* (B) MRSA (C) *S. hominis* and (D) *S. epidermidis*.


A
250.0000%
200.0000%
150.0000%
100.0000%
50.0000%
0.0000%
Survival percentage (%)
0 h
1/12 h
1/4 h
1/2 h
1 h
2 h
4 h
8 h
12 h
24 h
Time (h)
Compound 3
Compound 4
Compound 7
Control


B
160.0000%
140.0000%
120.0000%
100.0000%
80.0000%
60.0000%
40.0000%
20.0000%
0.0000%
Survival percentage (%)
0
1/12 h
1/6 h
1/2 h
1h
2h
4h
8h
12h
24h
Time (h)
Compound 3
Compound 4
Compound 7
Control



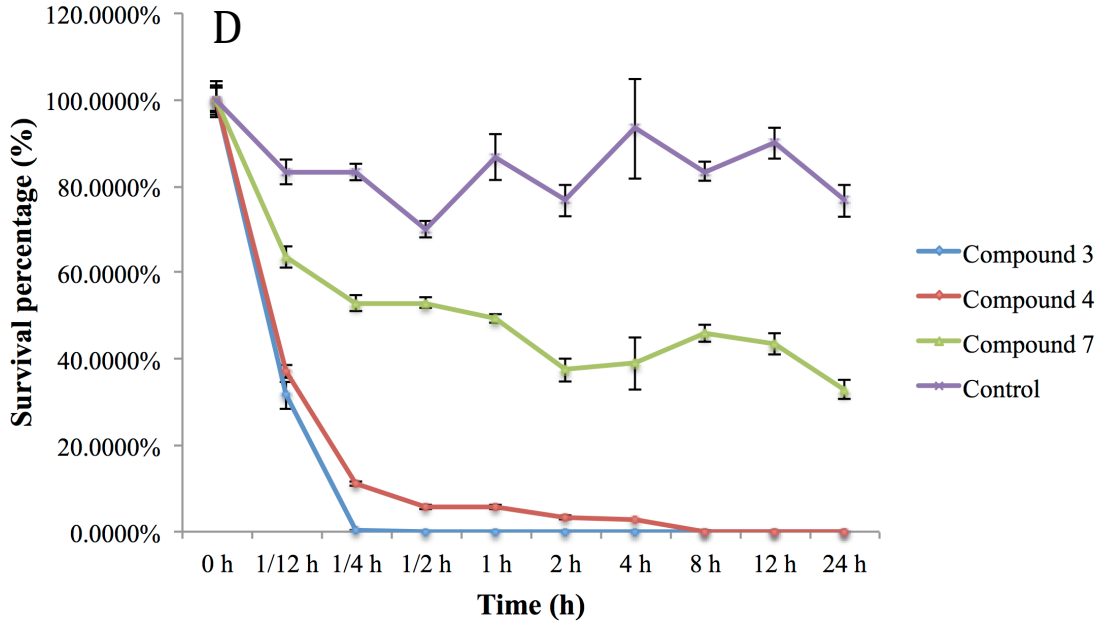


Fig. 5. Bactericidal activity of Compounds 3, 4, and 7 represented as percent survival. (A) *P. aeruginosa* (B) MRSA (C) *S. hominis* and (D) *S. epidermidis*.

Table 4. Percent Survival of *P. aeruginosa*

Time point	Compound 3	Compound 4	Compound 7	Control
0	100.0000%	100.0000%	100.0000%	100.0000%
1/12 h	22.1467%	43.3303%	108.3113%	86.4515%
1/4 h	23.9007%	34.6211%	114.8085%	131.4798%
1/2 h	26.7752%	50.5317%	98.2876%	111.7827%
1 h	28.5848%	51.4836%	141.4119%	120.4499%
2 h	44.3269%	71.7711%	119.1736%	120.4814%
4 h	17.6760%	36.8012%	92.8089%	118.1658%
8 h	0.0050%	17.5840%	103.2877%	168.8298%
12 h	0.0024%	2.2911%	97.9283%	218.6587%
24 h	0.0030%	0.0040%	120.6627%	222.4476%

Table 5. Percent Survival of MRSA

Time point	Compound 3	Compound 4	Compound 7	Control
0	100.0000%	100.0000%	100.0000%	100.0000%
1/12 h	6.9925%	66.1479%	40.6504%	82.3810%
1/4 h	3.8689%	86.7704%	84.0244%	87.2857%
1/2 h	2.6217%	92.2179%	72.8862%	77.7619%
1 h	2.4981%	64.7082%	54.1870%	77.7619%
2 h	2.0974%	63.4241%	49.6341%	77.7619%
4 h	1.5356%	61.3619%	29.3496%	65.0952%
8 h	0.4869%	55.7588%	20.7317%	58.7143%
12 h	0.4682%	42.4125%	18.8496%	58.7143%
24 h	0.1247%	39.5603%	5.6911%	46.0476%

Table 6. Percent Survival of *S. hominis*

Time point	Compound 3	Compound 4	Compound 7	Control
0	100.0000%	100.0000%	100.0000%	100.0000%
1/12 h	0.0155%	43.4132%	97.3926%	94.2029%
1/4 h	0.0009%	4.3114%	96.1656%	107.6812%
1/2 h	0.0005%	3.2934%	93.8650%	93.1884%
1 h	0.0003%	3.0689%	86.0429%	91.4493%
2 h	<0.00001%	0.9626%	66.7178%	79.7101%
4 h	<0.00001%	0.9626%	66.7178%	79.7101%
8 h	<0.00001%	0.4671%	64.7239%	70.7246%
12 h	<0.00001%	0.1692%	60.1227%	50.0000%
24 h	<0.00001%	0.0499%	44.4785%	26.8116%

Table 7. Percent Survival of *S. epidermidis*

Time point	Compound 3	Compound 4	Compound 7	Control
0	100.0000%	100.0000%	100.0000%	100.0000%
1/12 h	31.6667%	36.9231%	63.6042%	83.3333%
1/4 h	0.2125%	11.1538%	53.0035%	83.3333%
1/2 h	0.0096%	5.7692%	53.0035%	70.0000%
1 h	0.0010%	5.7692%	49.4700%	86.6667%
2 h	0.0004%	3.1923%	37.4558%	76.6667%
4 h	0.0004%	2.8846%	38.8693%	93.3333%
8 h	0.0002%	0.0846%	45.9364%	83.3333%
12 h	<0.00001%	0.0002%	43.4629%	90.0000%
24 h	<0.00001%	<0.00001%	32.8622%	76.6667%