Supporting Information

High Selective Performance of Rationally Designed Antimicrobial Peptides Based on Ponericin-W1

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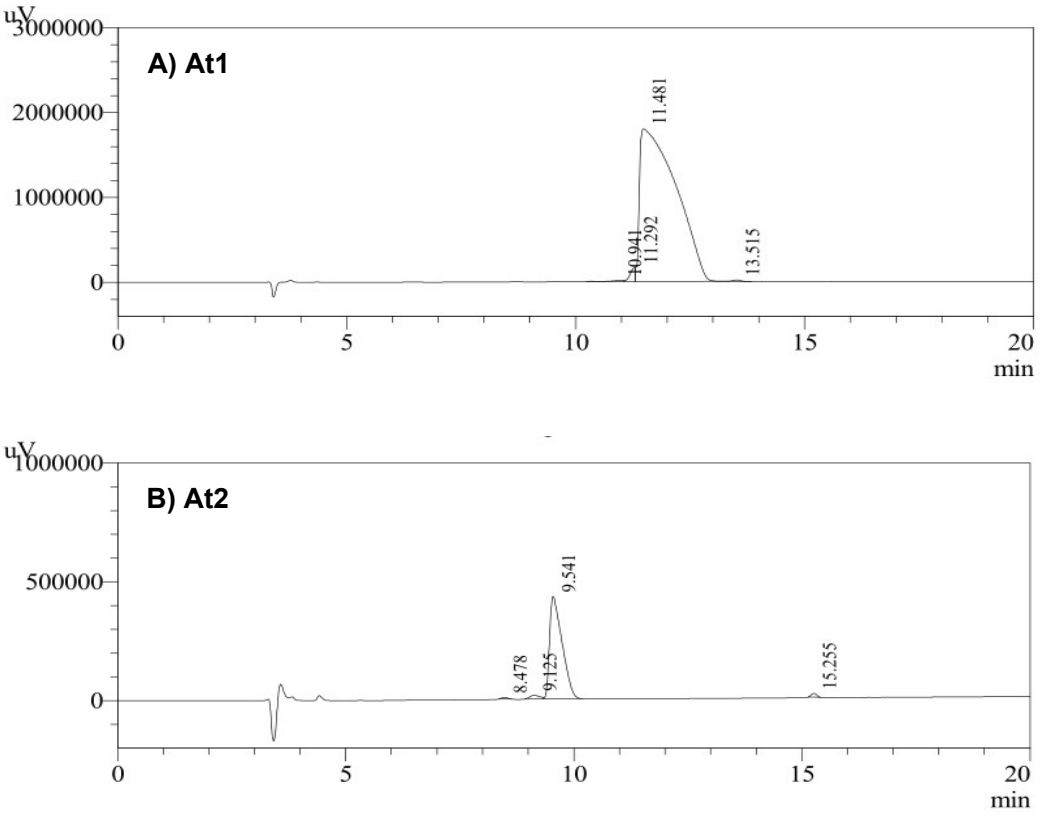

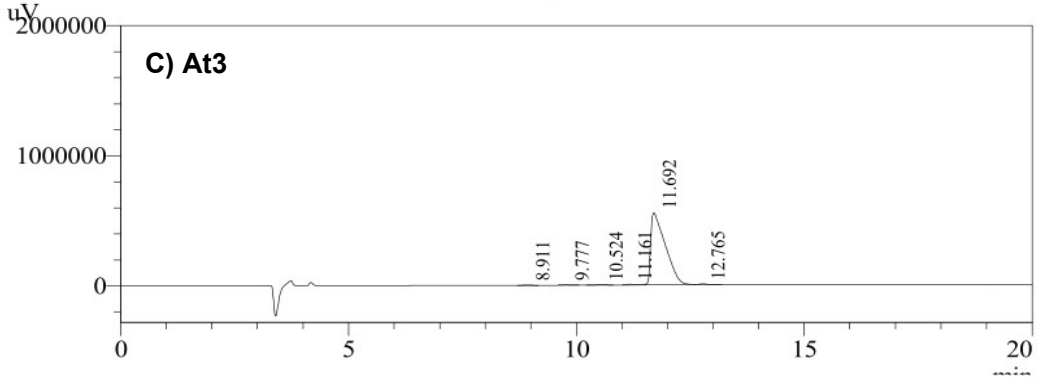
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2000000
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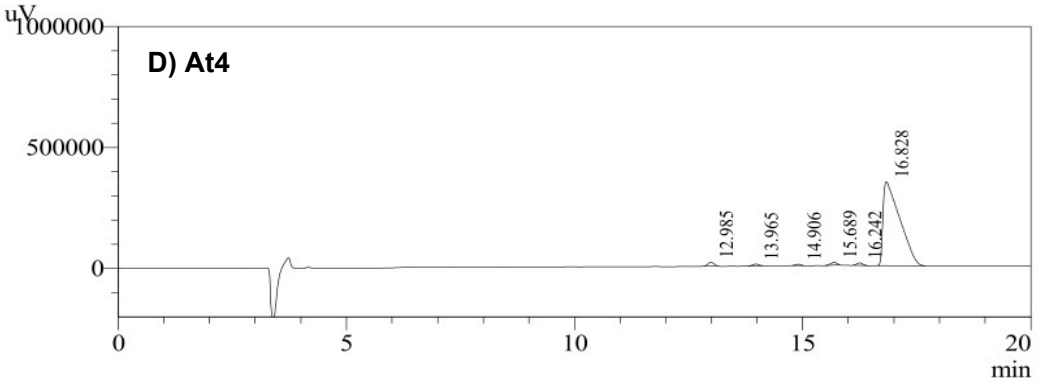
10.524
11.161
11.692
12.765
0
5
10
15
20


uV
1000000
500000
0
D) At4
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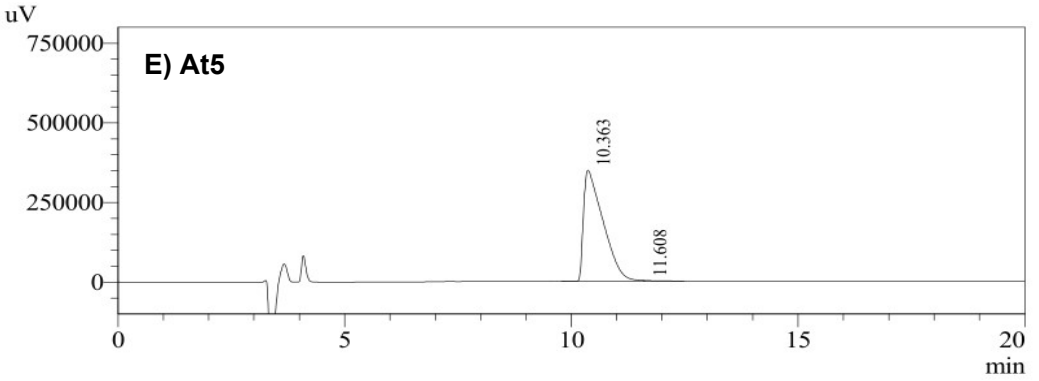
13.965
14.906
15.689
16.242
16.828
0
5
10
15
20
min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750000
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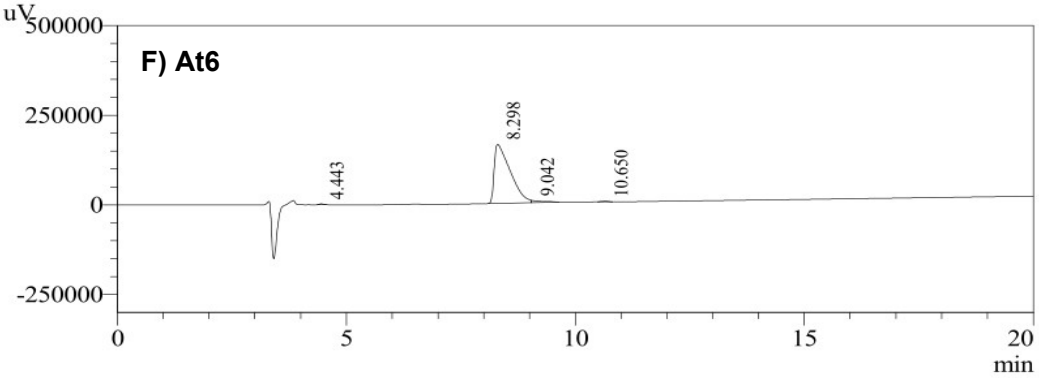
250000
0
E) At5
10.363
11.608
0
5
10
15
20
min


uV
500000
250000
0
-250000
F) At6
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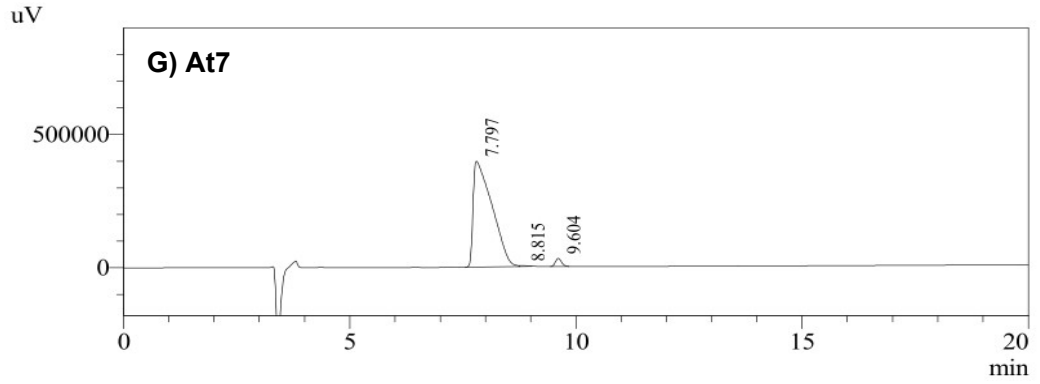
8.298
9.042
10.650
0
5
10
15
20
min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G) At7
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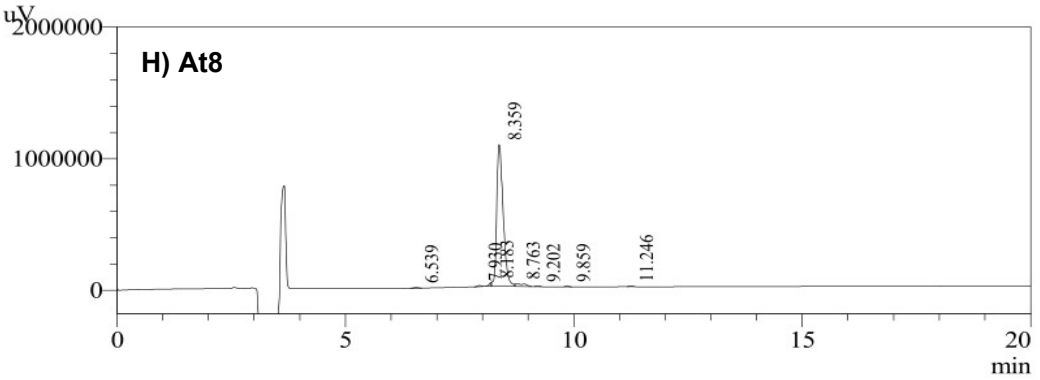
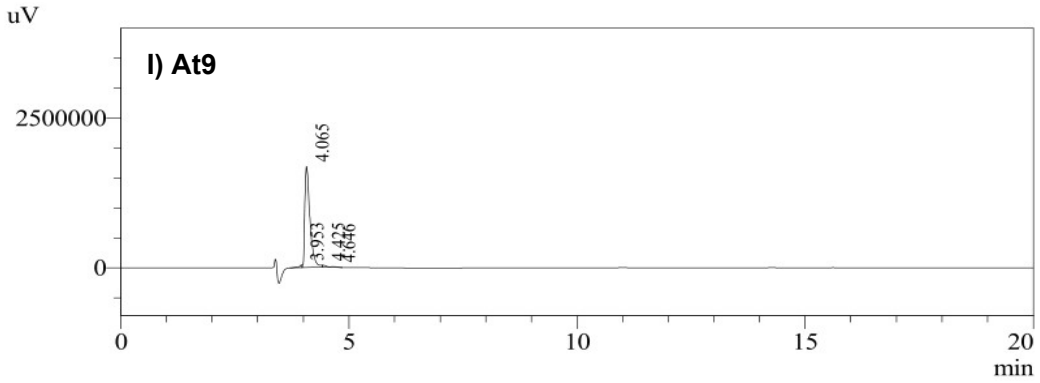
0
7.797
8.815
9.604
0
5
10
15
20
min


uV
2000000
H) At8
1000000
0
8.359
6.539
7.930
8.183
8.763
9.202
9.859
11.246
0
5
10
15
20
min


uV
I) At9
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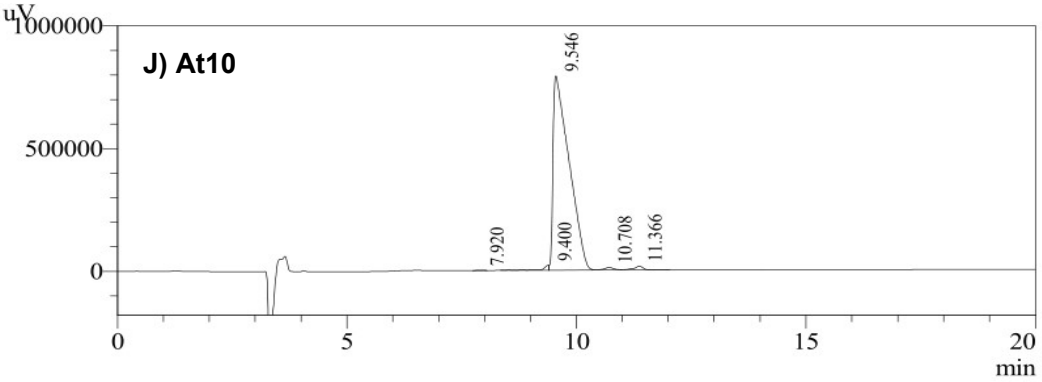
0
4.065
3.953
4.425
4.646
0
5
10
15
20
min


uV
1000000
J) At10
500000
0
9.546
7.920
9.400
10.708
11.366
0
5
10
15
20
min

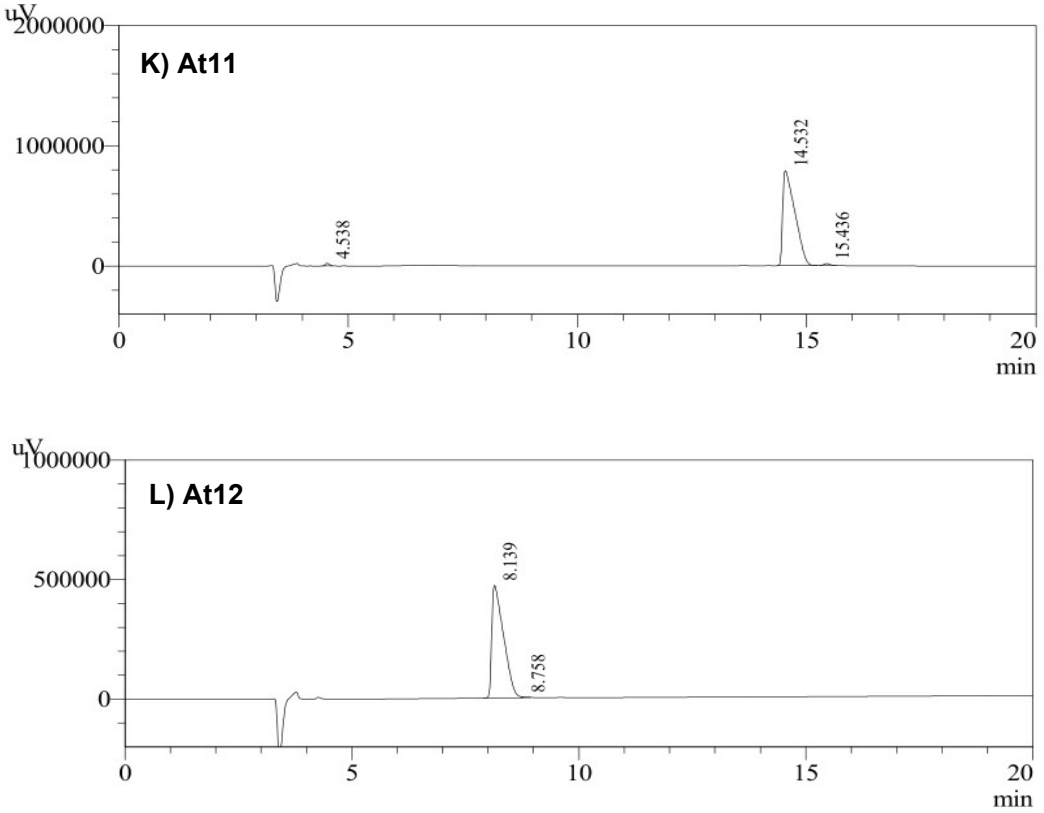
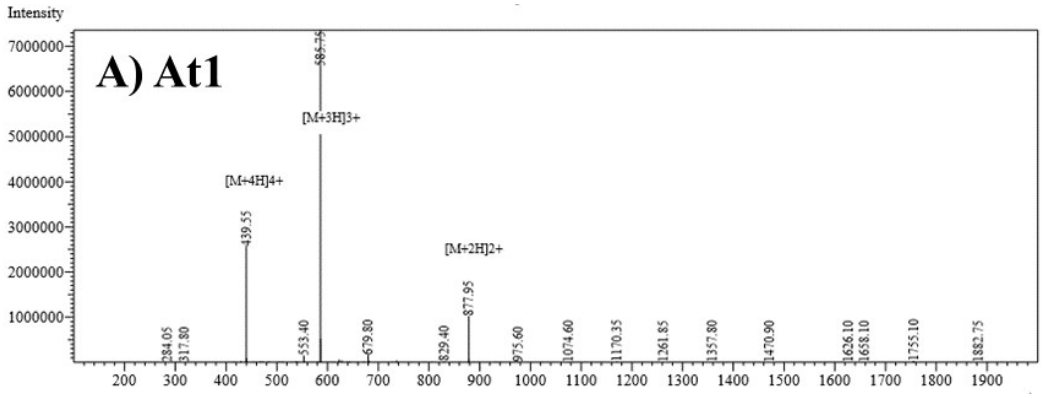
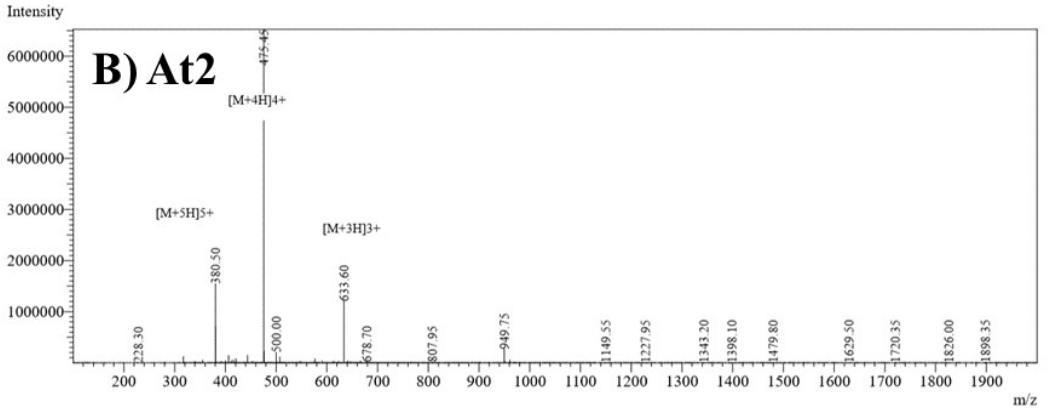
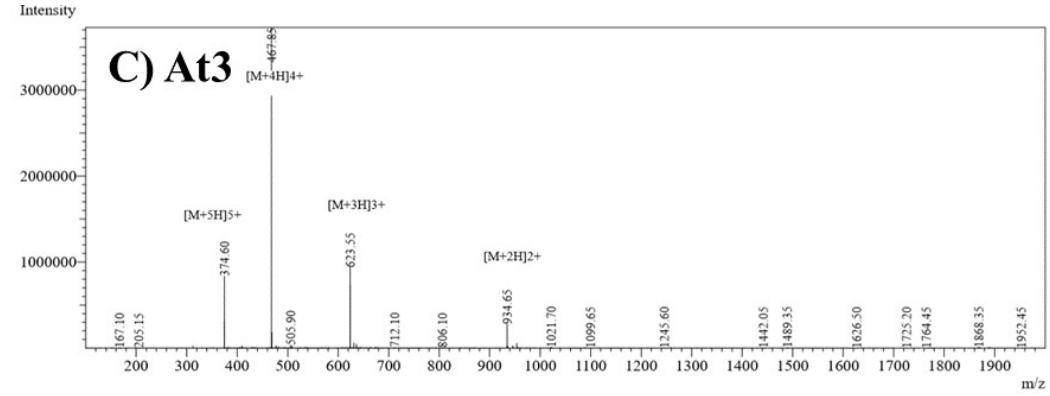
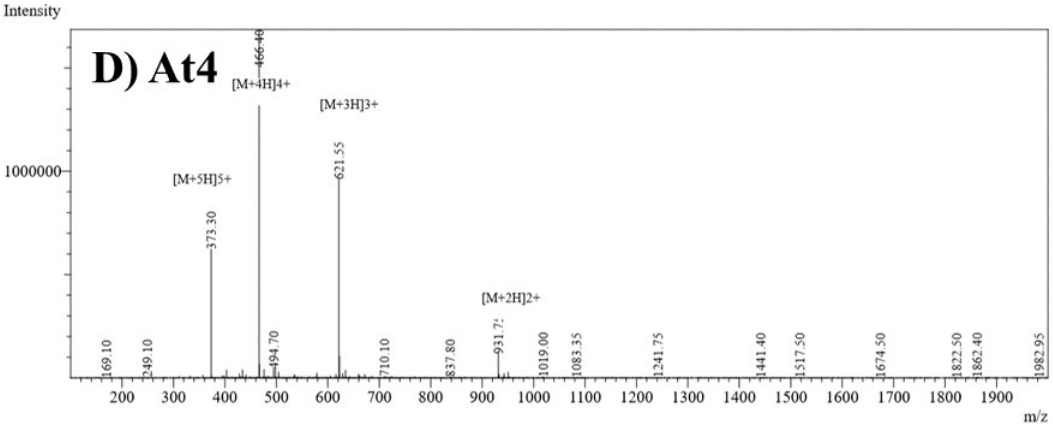


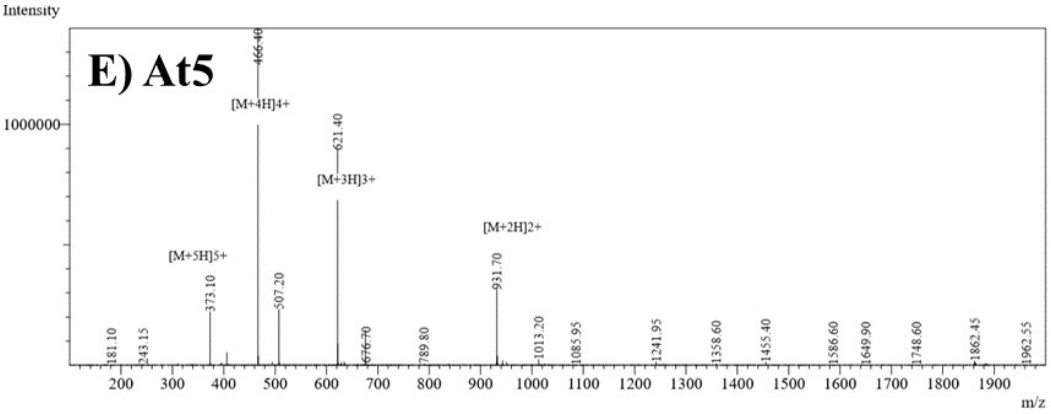
Figure S1. HPLC chromatograms of At1-At12 peptides. HPLC conditions: The peptide concentration was fixed at 1 mg/mL. Analytical column type: SHIMADZU Inertsil ODS-SP (4.6 x 250 mm x 5 μm). Eluent A (0.1% trifluoroacetic in water) and eluent B (0.1% trifluoroacetic in acetonitrile). The flow rate was 1 mL/min and the UV detector was set at 214 nm.

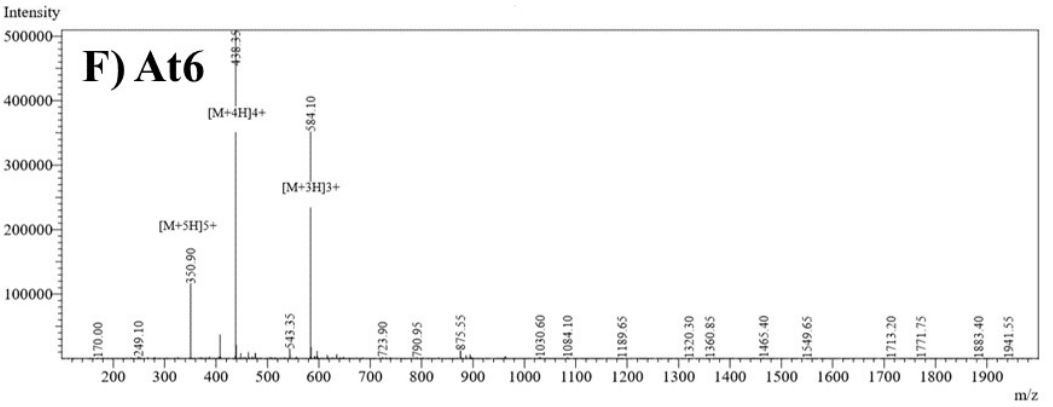


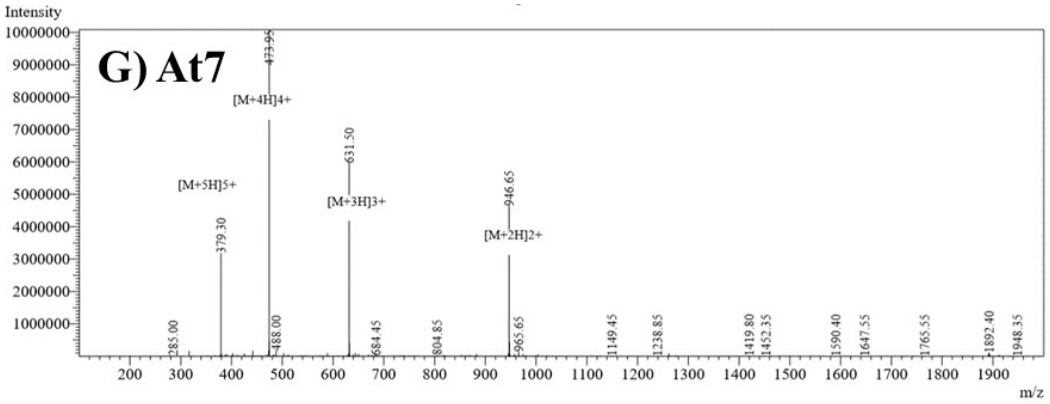

Intensity
B) At2
[M+4H]4+
[M+5H]5+
[M+3H]3+
475.45
380.50
633.60
228.30
500.00
678.70
807.95
949.75
1149.55
1227.95
1343.20
1398.10
1479.80
1639.50
1720.35
1826.00
1898.35
m/z

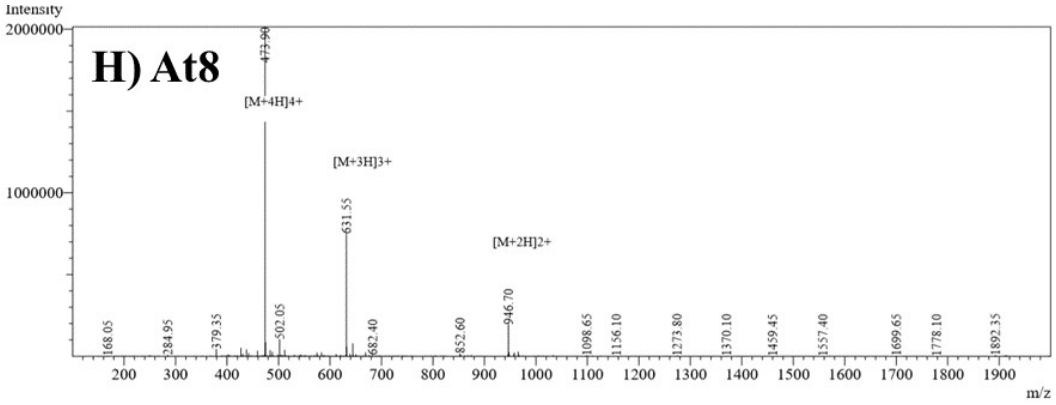

Intensity
C) At3
[M+4H]4+
[M+5H]5+
[M+3H]3+
[M+2H]2+
467.85
374.60
623.55
934.65
167.10
205.15
505.90
712.10
806.10
1021.70
1099.65
1245.60
1442.05
1489.35
1626.50
1725.20
1764.45
1868.35
1952.45
m/z

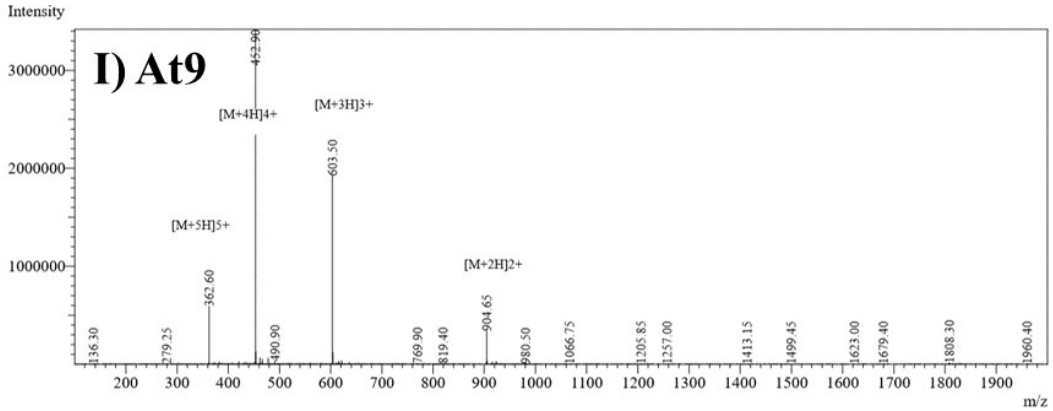

Intensity
D) At4
[M+4H]4+
[M+3H]3+
[M+5H]5+
[M+2H]2+
466.48
621.55
373.30
931.7
169.10
249.10
494.70
710.10
837.80
1019.00
1083.35
1241.75
1441.40
1517.50
1674.50
1822.50
1862.40
1982.95
m/z


Intensity
E) At5
[M+4H]4+
[M+3H]3+
[M+5H]5+
[M+2H]2+
466.48
621.40
373.10
507.20
931.70
181.10
243.15
676.70
789.80
1013.20
1085.95
1241.95
1358.60
1455.40
1586.60
1649.90
1748.60
1862.45
1962.55
m/z


F) At6
Intensity
[M+4H]4+
[M+3H]3+
[M+5H]5+
438.35
584.10
350.90
m/z


G) At7
Intensity
[M+4H]4+
[M+3H]3+
[M+2H]2+
[M+5H]5+
473.95
631.50
946.65
379.30
m/z


H) At8
Intensity
[M+4H]4+
[M+3H]3+
[M+2H]2+
473.90
631.55
946.70
m/z


I) At9
Intensity
[M+4H]4+
[M+3H]3+
[M+5H]5+
[M+2H]2+
452.90
603.50
362.60
904.65
m/z

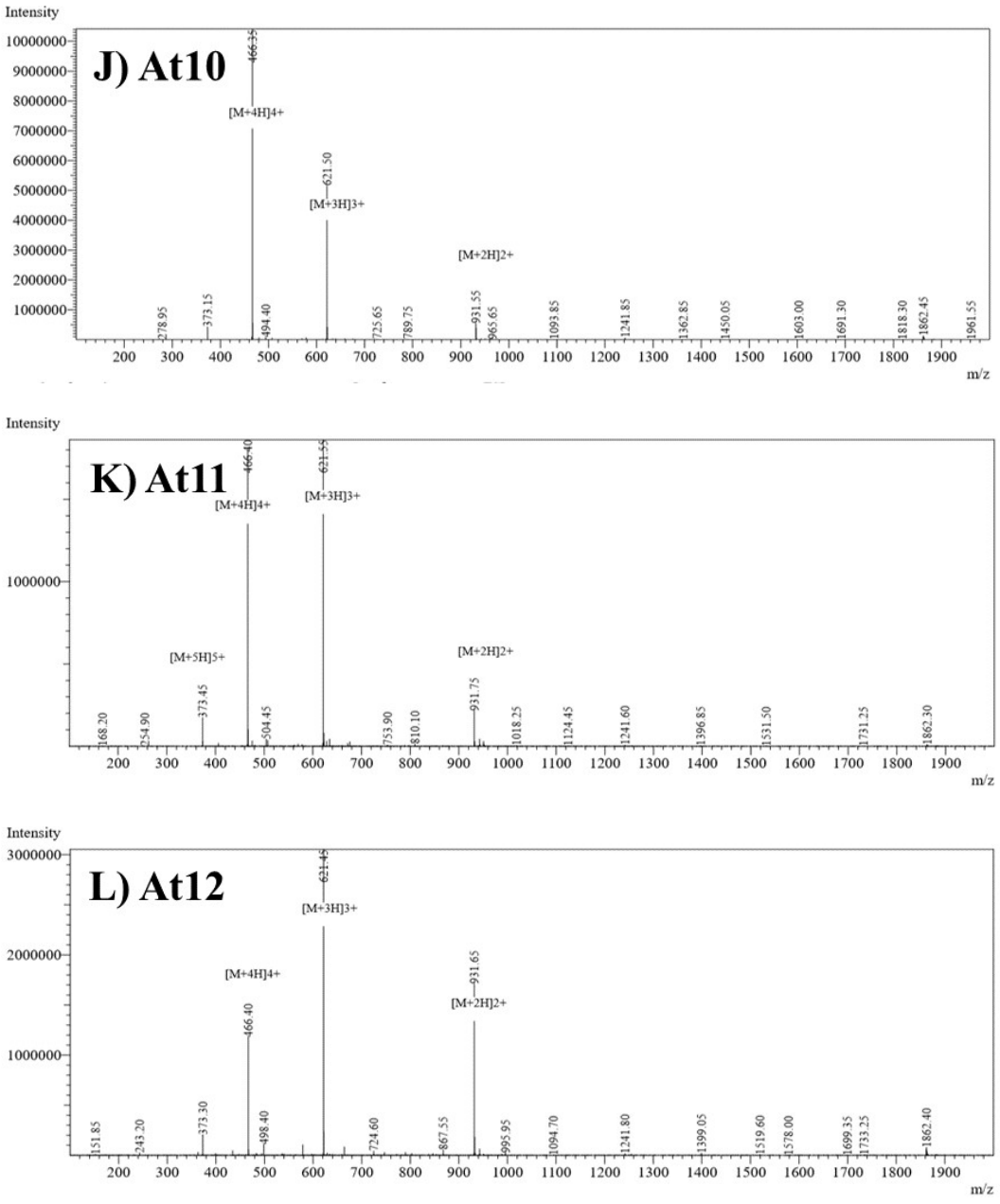


Figure S2. Mass spectrum of peptides.

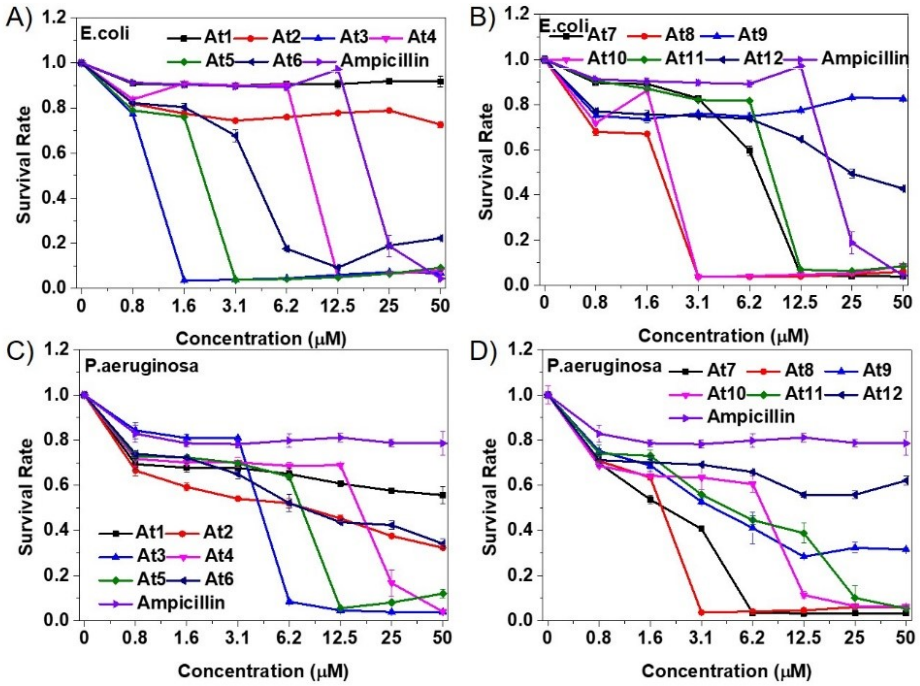


Figure S3. The MIC of antimicrobial peptides to gram-negative bacteria *E. coli* (**A-B**) and *P. aeruginosa* (**C-D**).

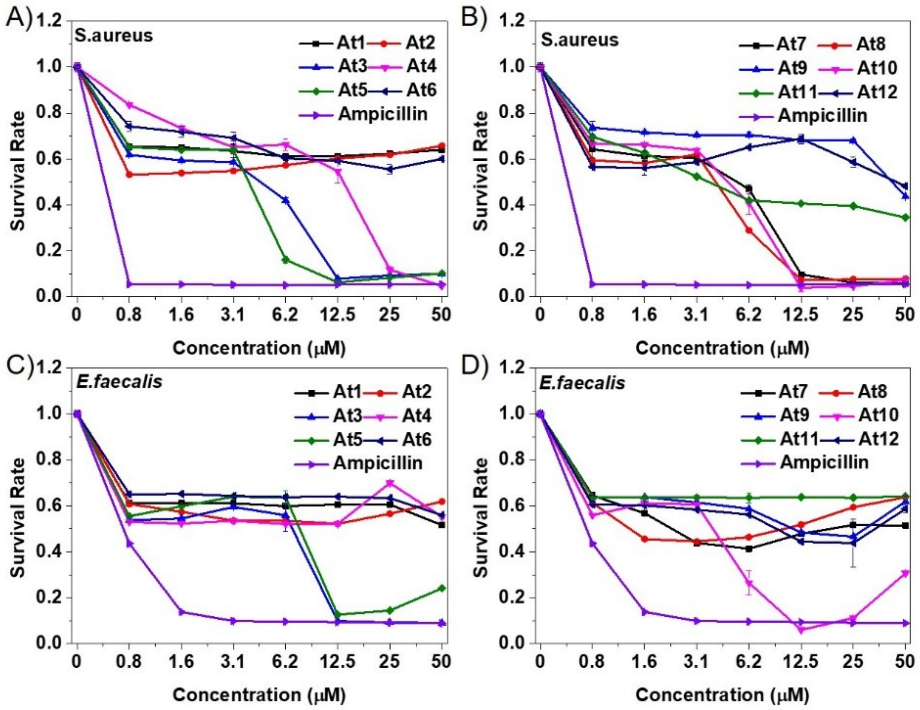


Figure S4. Bacteria The MIC of antimicrobial peptides to gram-positive bacteria *S. aureus* (**A-B**) and *E. faecalis* (**C-D**).

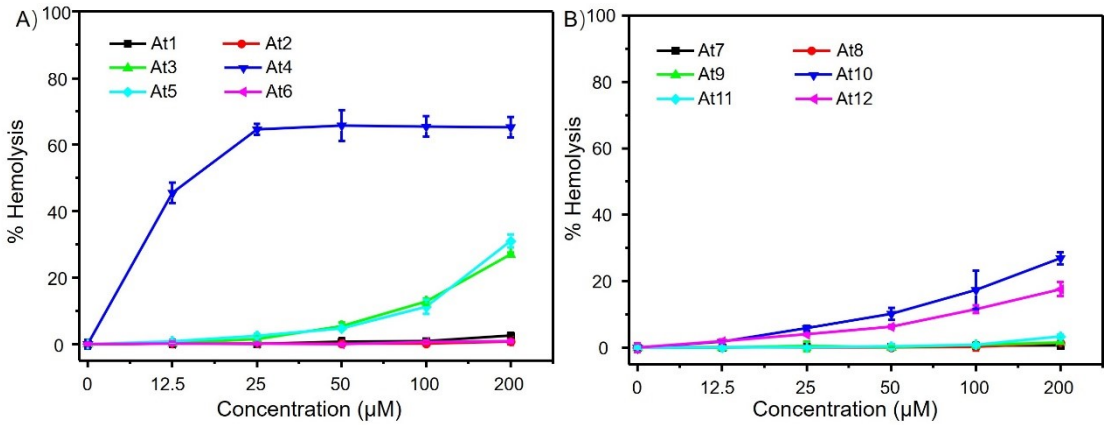


Figure S5. Hemolytic activities of peptides (At1-At12). Human red blood cells (hRBCs) were treated with different concentrations of peptides and incubated for 1 h at 37 ℃. Then measure the absorbance at OD_{540nm}.

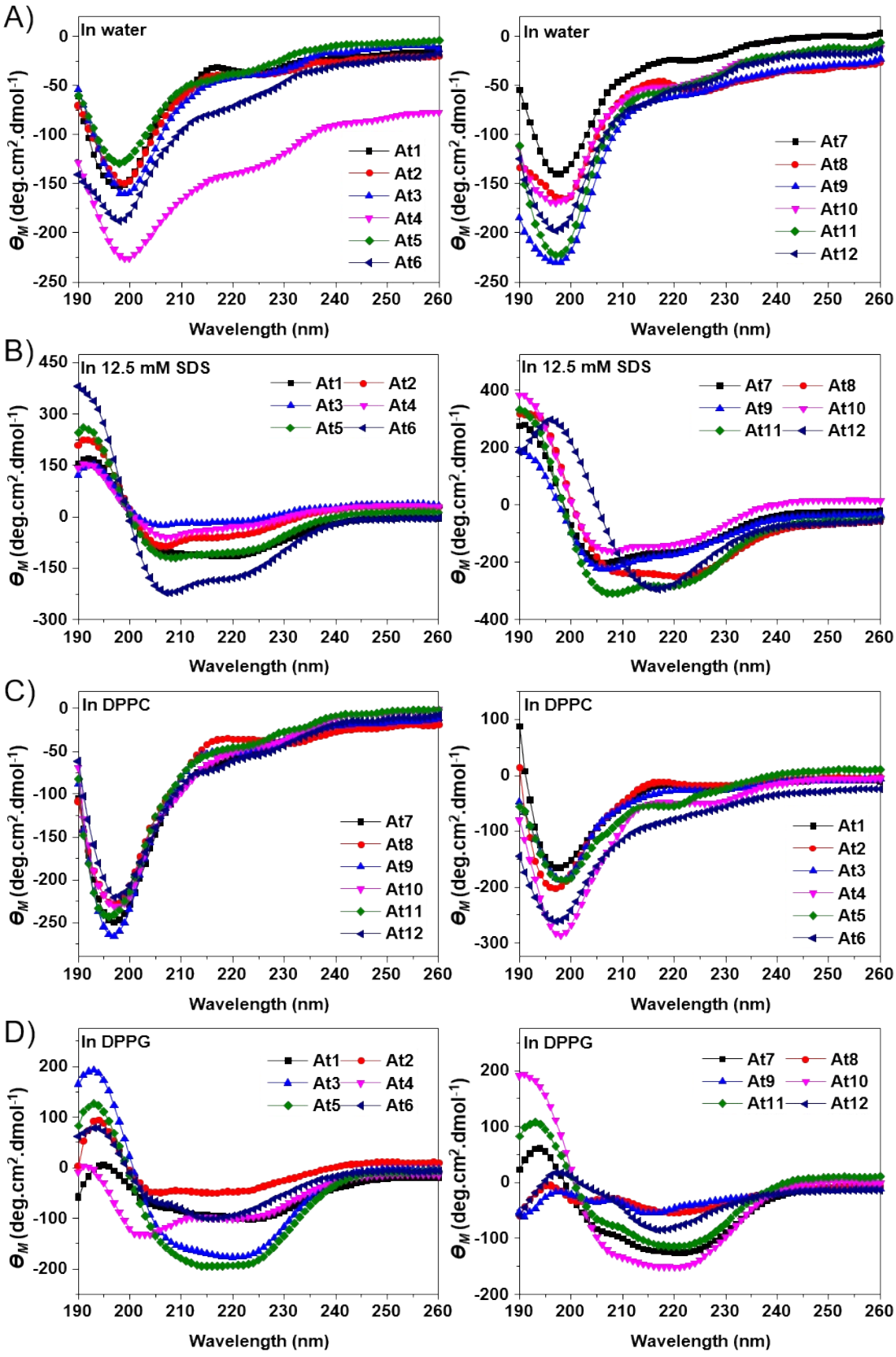


Figure S6. CD spectra of peptides in (A) water, (B) zwitterionic DPPC SUVs (0.25 mg/mL) solution, (C) 25 mM SDS and (D) negatively charged DPPG SUVs (0.25 mg/mL).

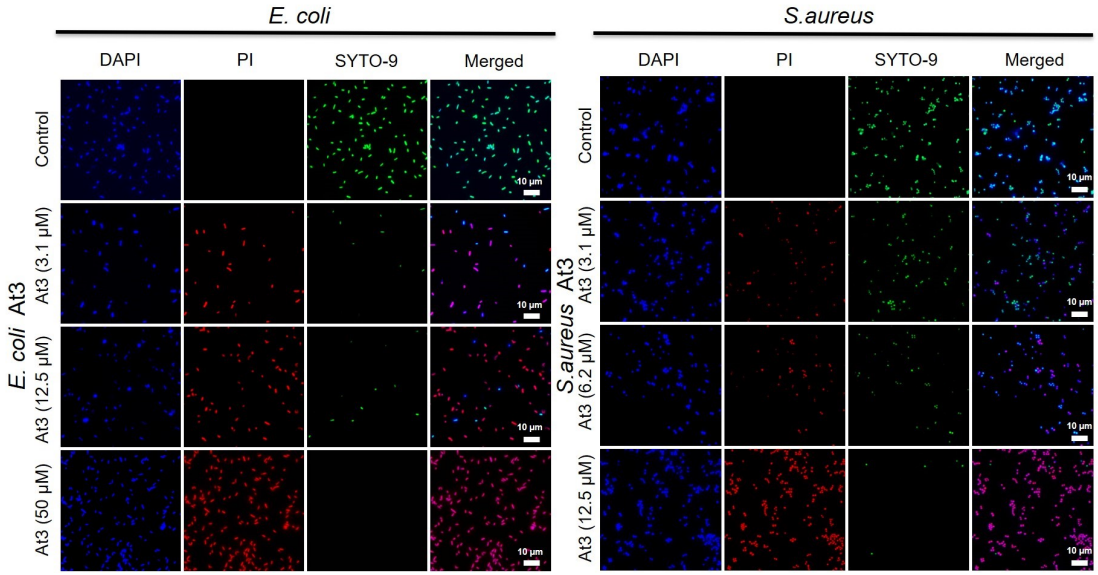


Figure S7. Live/Dead staining assay of *E. coli* and *S. aureus* before and after the treatment of At3 peptides at different concentrations for 2 h at 37 ℃.

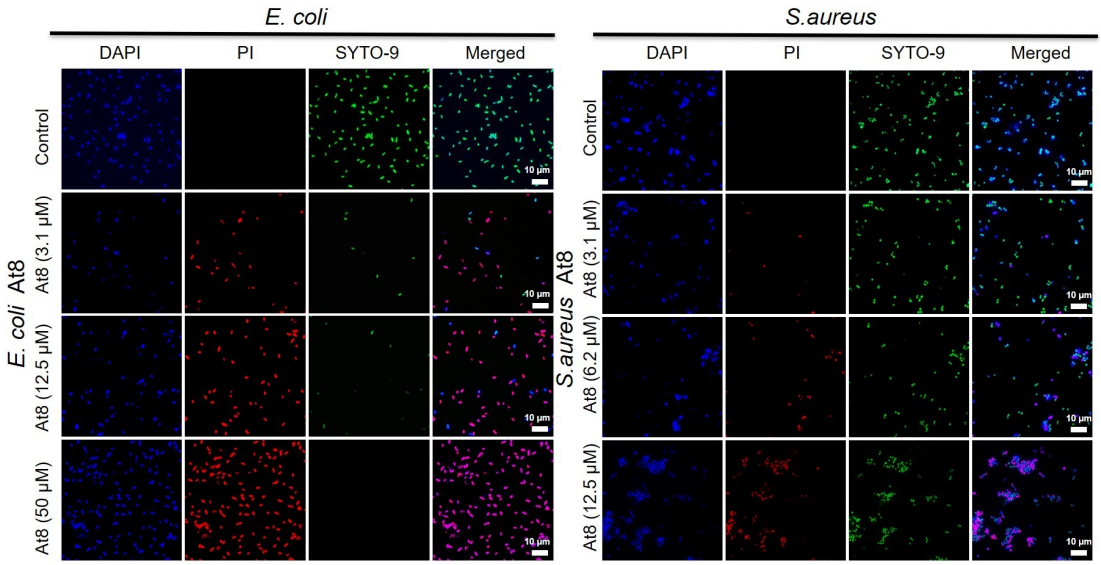


Figure S8. Live/Dead staining assay of *E. coli* and *S. aureus* before and after the treatment of At8 peptides at different concentrations for 2 h at 37 ℃.

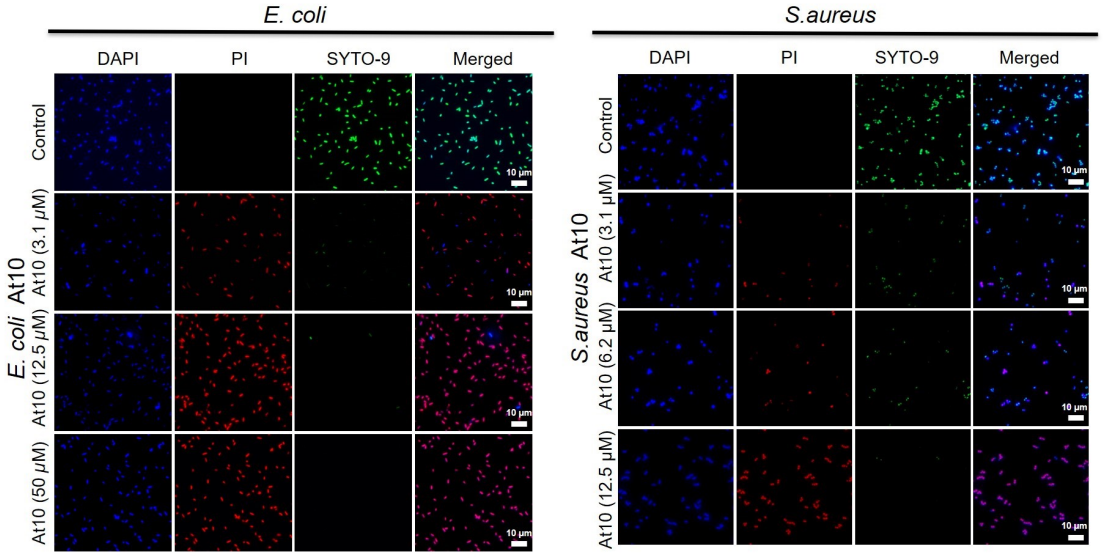


Figure S9. Live/Dead staining assay of *E. coli* and *S. aureus* before and after the treatment of At10 peptides at different concentrations for 2 h at 37 ℃.

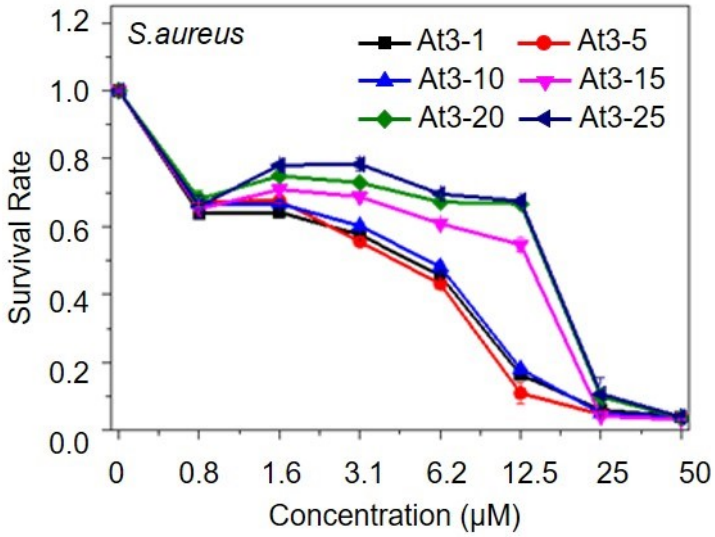


Figure S10. Development of antimicrobial resistance (AMR). S. aureus bacteria treated with At3 peptide (25 passages).