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Antibacterial Activity of Specialized Biomolecules

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Introduction

Bacterial growth can be inhibited by antimicrobial agents, causing disruption of vital cellular functions resulting in rapid cell death. Typically, these agents act at the level of the bacterial membrane, which is a crucial structure for cell survival. Currently, there is a vast array of antimicrobial biomolecules. For many years, the most widely used have been the antibiotics. However, their excessive consumption has led to an alarmingly high resistance development by bacterial pathogens, raising a serious global public-health problem. Hence, the interest in the research for novel alternatives to antibiotics has been growing. Natural products are becoming very promising as antimicrobial agents, being considered safe and environmentally friendly. Here, we envisage the evaluation of the antimicrobial efficacy of antimicrobial peptides (AMPs), namely LL37 and pexiganan, and essential oils (EOs), tea tree oil (TTO), cinnamon leaf oil (CLO) and niaouli oil (NO), against four bacteria commonly associated to nosocomial infections: *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Escherichia coli* and *Pseudomonas aeruginosa*. The antibiotic vancomycin and silver nanoparticles (AgNPs) were used as control compounds for comparison purposes.

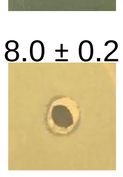
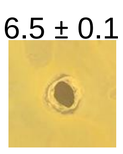
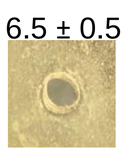
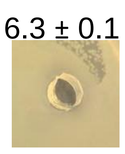
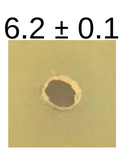
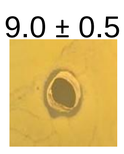
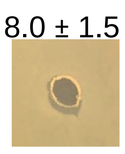
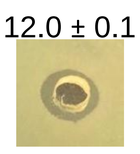
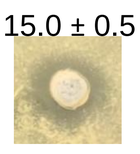
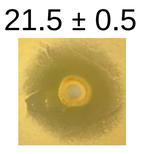
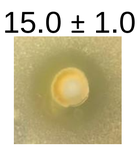
Antimicrobial Solutions Preparation

Antimicrobial agents	Solvent	Concentrations
AgNPs	Distilled water (dH_2O)	5000-1.95 µg/mL
Vancomycin	dH_2O	2000-1.95 µg/mL
LL37	Phosphate buffered saline solution (PBS)	1000-0.98 µg/mL
Pexiganan	dH_2O	1000-0.98 µg/mL
TTO	Mueller Hinton broth (MHB)	500-0.18 mg/mL
CLO	MHB	500-0.18 mg/mL
NO	MHB	500-0.18 mg/mL

Antimicrobial Action

1) Agar-Well Diffusion Assay

Initial Bacteria Concentration: $2x10^6$ CFUs/mL in TSB
For the agents, the highest concentration was used.

Antimicrobial agents	ZoI Diameter (mm)			
	S. aureus	***S. epidermidis***	***E. coli***	***P. aeruginosa***
AgNPs	11.5 ± 1.7 	10.6 ± 0.6 	8.8 ± 0.5 	8.8 ± 3.0 
Vancomycin	22.5 ± 0.5 	22.5 ± 0.5 	8.0 ± 0.1 	8.0 ± 0.2 
LL37	6.5 ± 0.1 	6.5 ± 0.5 	6.3 ± 0.1 	6.2 ± 0.1 
Pexiganan	9.0 ± 0.5 	12.2 ± 0.6 	8.0 ± 1.5 	12.0 ± 0.1 
TTO	20.2 ± 0.1 	15.0 ± 0.5 	15.5 ± 0.5 	13.3 ± 0.3 
CLO	21.5 ± 0.5 	15.0 ± 1.0 	15.0 ± 1.9 	15.0 ± 0.6 
NO	14.7 ± 0.4 	10.0 ± 0.5 	11.5 ± 0.5 	6.8 ± 0.5 

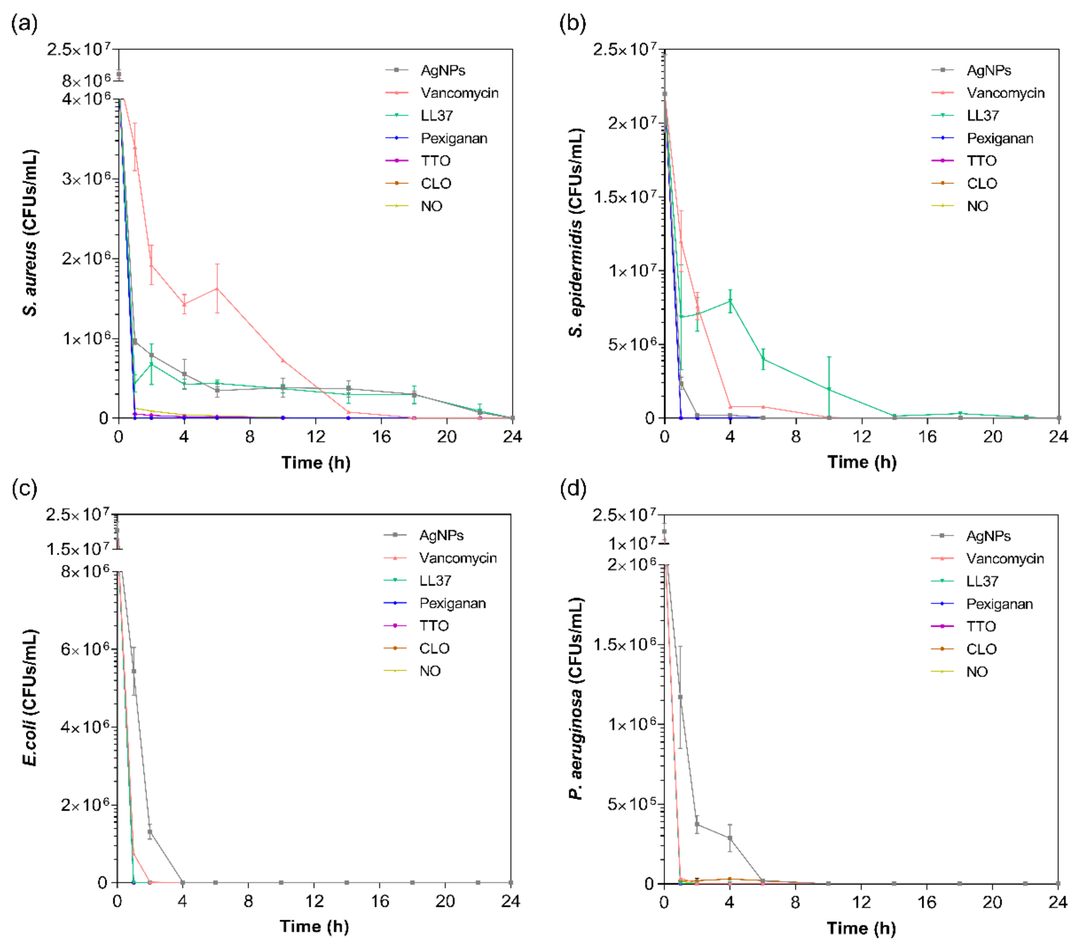
2) Minimum Inhibitory Concentrations (MICs)

Initial Bacteria Concentration: $2x10^7$ CFUs/mL in MHB

Antimicrobial agents	MICs			
	S. aureus	***S. epidermidis***	***E. coli***	***P. aeruginosa***
AgNPs	4000.0 µg/mL	4000.0 µg/mL	4000.0 µg/mL	1250.0 µg/mL
Vancomycin	7.8 µg/mL	7.8 µg/mL	1000.0 µg/mL	1000.0 µg/mL
LL37	500.0 µg/mL	500.0 µg/mL	125.0 µg/mL	250.0 µg/mL
Pexiganan	31.3 µg/mL	7.8 µg/mL	62.5 µg/mL	31.3 µg/mL
TTO	67.1 mg/mL	179.0 mg/mL	33.6 mg/mL	268.5 mg/mL
CLO	26.2 mg/mL	26.2 mg/mL	19.7 mg/mL	39.3 mg/mL
NO	137.0 mg/mL	182.6 mg/mL	137.0 mg/mL	365.2 mg/mL

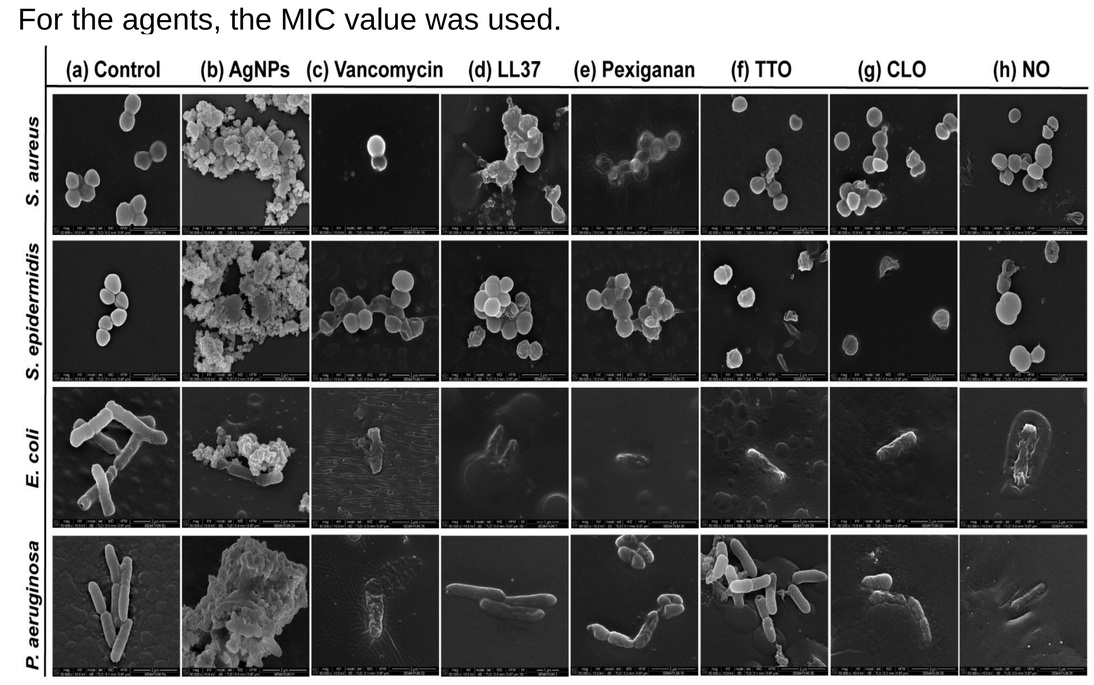
3) Kill-time Analysis: Bacteria Viability

Initial Bacteria Concentration: $2x10^7$ CFUs/mL in MHB
For the agents, the MIC value was used.



4) Cell-Wall Disruption: Mechanisms of Action (SEM observations)

Initial Bacteria Concentration: $2x10^7$ CFUs/mL in MHB
For the agents, the MIC value was used.



Conclusions: All agents were effective against the selected bacteria. Interestingly, the AgNPs required a higher concentration (4000–1250 µg/mL) to induce the same effects as the AMPs (500–7.8 µg/mL). Pexiganan was the most effective biomolecule.

For more details please refer to **DOI: 10.3390/antibiotics9060314**

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