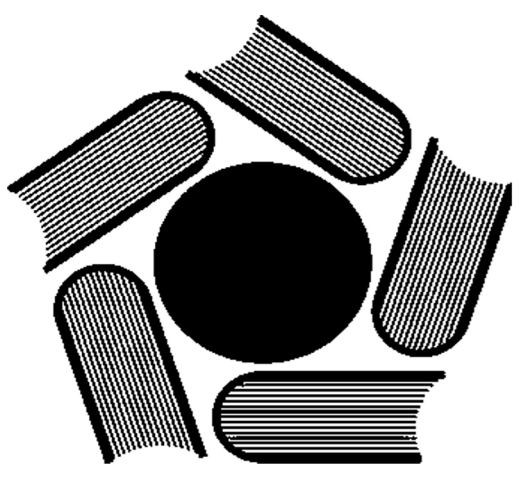


Defensive secretions of millipedes *Megaphyllum unilineatum* (C. L. Koch, 1838), *Pachyiulus hungaricus* (Karsch, 1881) and *Cylindroiulus boleti* (C. L. Koch, 1847) (Diplopoda, Julida) as antimicrobial agents in the inhibition of biofilms of *Pseudomonas aeruginosa* PAO1 and *Staphylococcus aureus*



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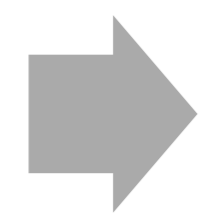
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INTRODUCTION

IN RECENT YEARS: increased resistance of microorganisms to antibiotics



IMPERATIVE: discovery of new antimicrobial agents

- BACTERIAL BIOFILMS are highly resistant to antimicrobial agents which is serious global health concern
- plants and animals are a valuable source of natural biologically active products
- good basis for finding new antimicrobial and antibiofilm agents

OBJECTIVES

Determine the inhibition and degradation of the formed biofilm of *P. aeruginosa* PAO1 and *S. aureus* by defense secretions of selected millipede species from the family Julidae as well as to determine their antimicrobial activity by MTT assay.

MILLIPEDES (DIPLOPODA)

- complex defense against predators
- various chemical compounds
- showed antimicrobial activity
- ORDER JULIDA

Megaphyllum unilineatum (MUN)

Pachyiulus hungaricus (PHU)

Cylindroiulus boleti (CBO)

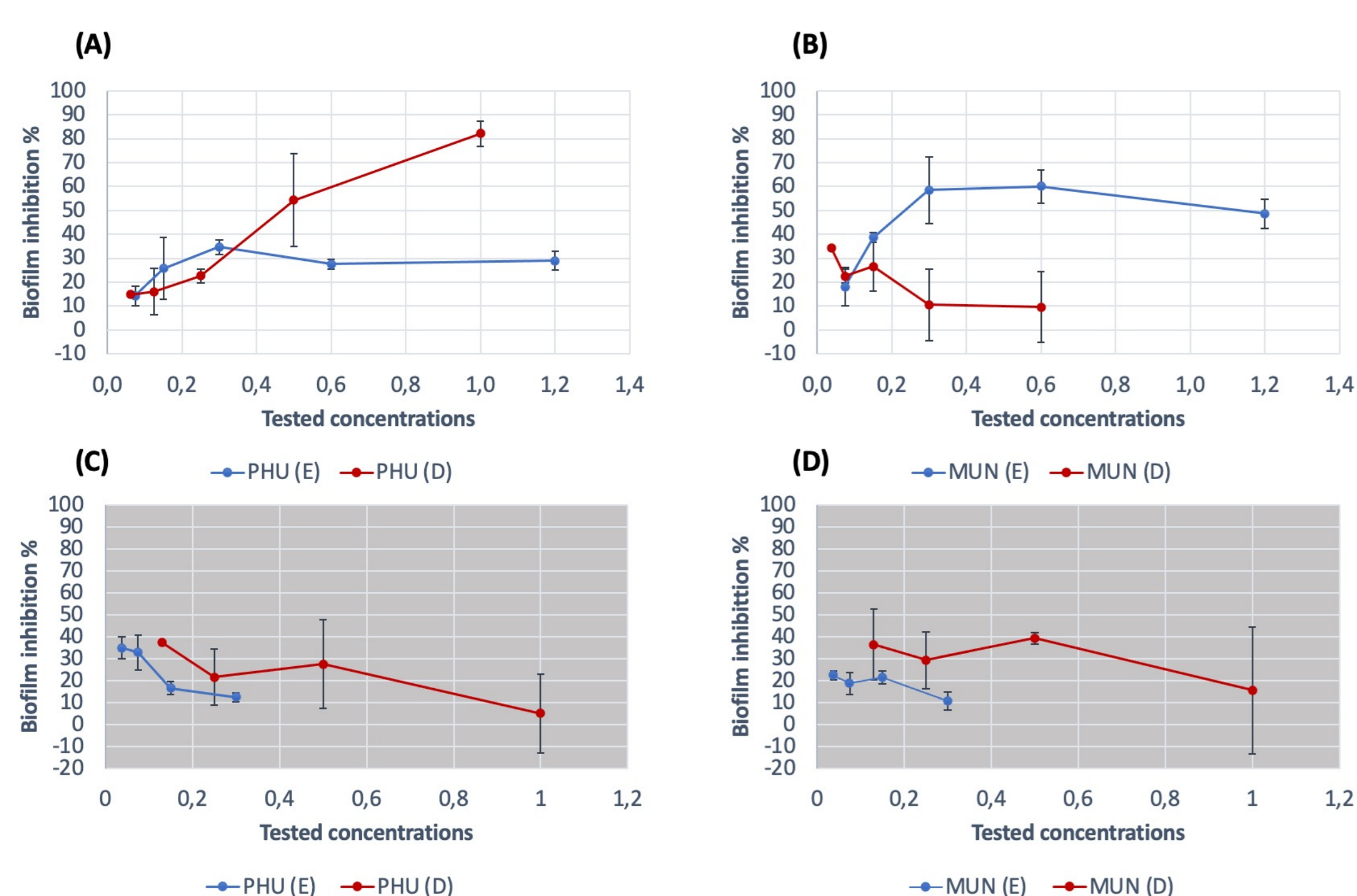
METHOD / DESIGN

BIOFILM FORMATION was quantified by the crystal violet staining method

ANTIMICROBIAL ACTIVITY was examined using the broth dilution minimum inhibitory concentration (MIC) test.

DMSO vs ETHANOL

- Inhibition of biofilm formation of defensive secretions (A) PHU and (B) MUN and degradation of the already formed *P. aeruginosa* PAO1 biofilm of (C) PHU and (D) MUN; both ethanol (E) and DMSO (D) extracts.
- DMSO extracts showed a stronger antibiofilm effect except in case B, where ethanolic MUN extract showed a stronger effect in biofilm inhibition.



RESULTS

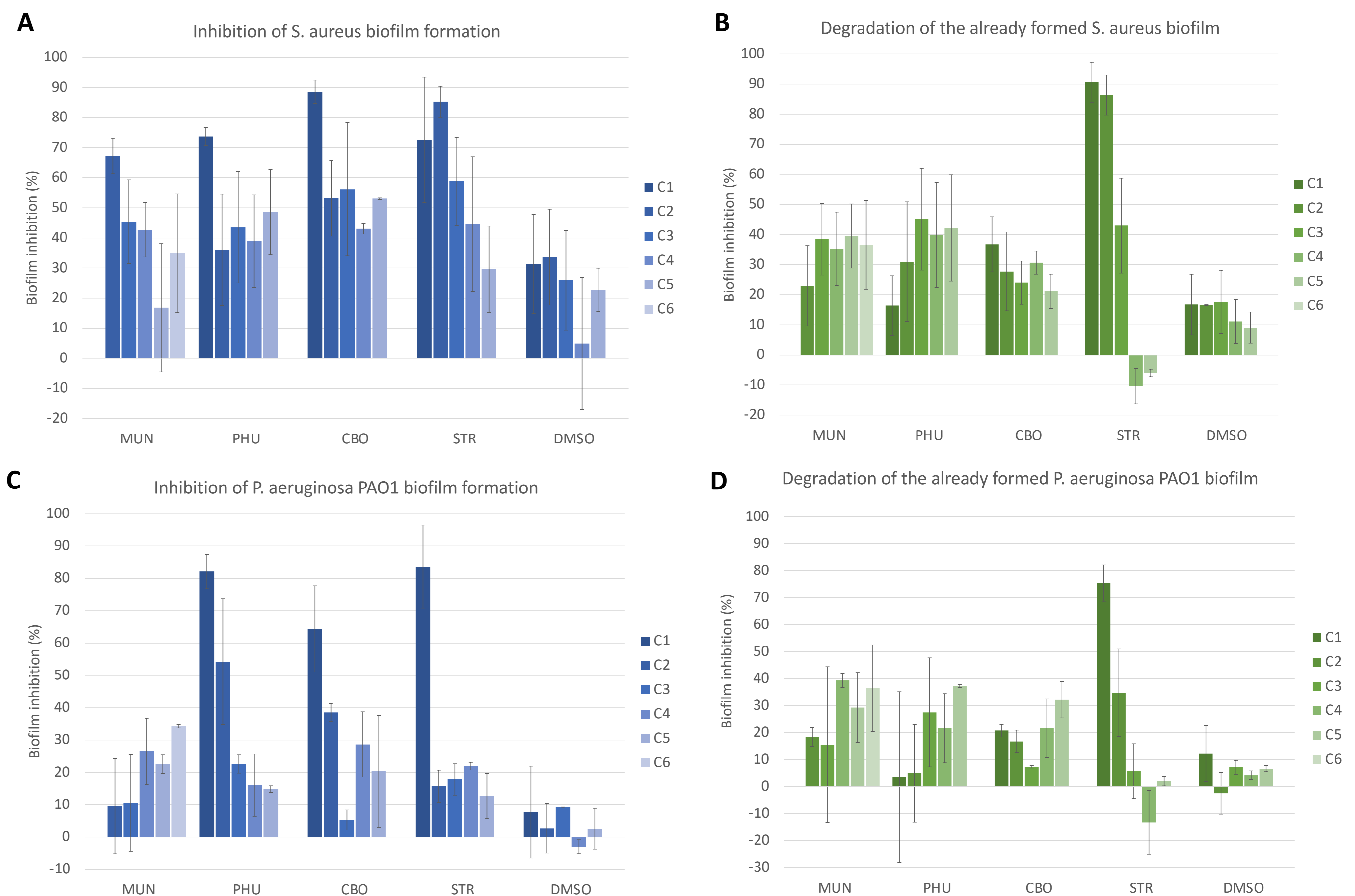
ANTIMICROBIAL ACTIVITY

- Defensive secretions showed a better antimicrobial activity against *S. aureus* compared to *P. aeruginosa* PAO1, which is most likely due to the high resistance of this bacterial strain.

mg/ml	<i>S. aureus</i>		<i>P. aeruginosa</i> (PAO1)	
	MIC	MBC	MIC	MBC
MUN	0.03	0.06	>1	>1
PHU	0.06	0.12	1	>1
CBO	0.06	0.12	1	>1
Streptomycin	0.006	0.012	0.006	0.012
DMSO	>2.5%	>2.5%	>2.5%	>2.5%

Minimum inhibitory concentrations - MIC and Minimum bactericidal concentrations - MBC on *S. aureus* and *P. aeruginosa* PAO1 bacterial strains, presented in mg/mL.

ANTIBIOFILM ACTIVITY



(A) Inhibition of *S. aureus* biofilm formation. Double dilutions were tested with the 2xMIC highest concentration tested (C1). (B) Degradation of the already formed *S. aureus* biofilm. Double dilutions were tested with the 4xMIC highest concentration tested (C1). (C) Inhibition of *P. aeruginosa* PAO1 biofilm formation. Double dilutions were tested with the highest concentration tested (C1) for MUN: 0.5 mg/ml, PHU and CBO: 1 mg/ml, DMSO: 1,25 % and streptomycin: 0,013 mg/ml. (D) Degradation of the already formed *P. aeruginosa* PAO1 biofilm. Double dilutions were tested with the highest concentration tested (C1) for MUN: 1 mg/ml, PHU and CBO: 2 mg/ml, DMSO: 2,5 % and streptomycin: 0,025 mg/ml.

- The antibiofilm effect was observed in all tested defense secretions and was more pronounced against *S. aureus* than against *P. aeruginosa* PAO1.
- The strongest biofilm inhibition of *S. aureus* was at the highest tested concentrations (2 × MIC) while degradation of already formed biofilm was shown at lower tested concentrations (MIC/4)
- The strongest inhibition of *P. aeruginosa* PAO1 biofilm formation was observed at the highest tested concentrations for PHU (82 and 54%), and CBO (64.3 and 38.5) while MUN had the strongest activity at the lowest tested concentration of 0.06 mg/mL (34.3%).
- All examined defense secretions had similar degradation activity of *P. aeruginosa* PAO1 biofilm with stronger activity at lower tested concentrations (about 30%).

CONCLUSIONS

The defense secretions of MBO, PHU, and CBO show a good basis for further investigations of their use as antimicrobial agents, especially against *S. aureus*.