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Effect of ethyl acetate extracts of the roots and aerial parts of *Geum urbanum* L. on the phenotype inhibition of the *Pseudomonas aeruginosa* Las/RhI quorum sensing system and their antioxidant activity

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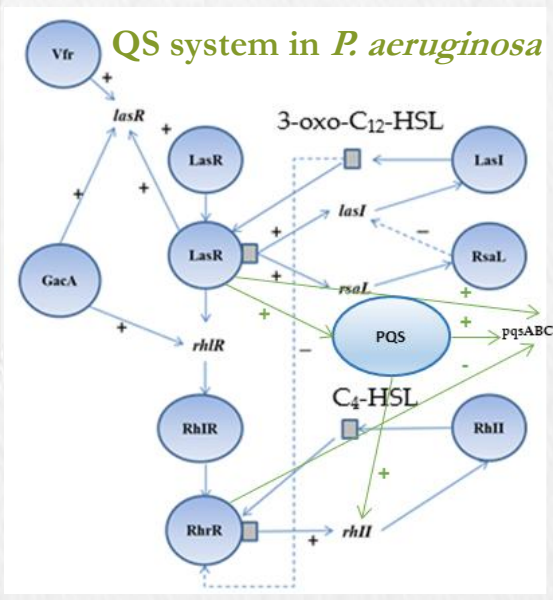
Abstract: The opportunistic pathogen *Pseudomonas aeruginosa* causes nosocomial infections affecting the urinary system, respiratory tract, etc. It can lead to bacteremia and sepsis, especially in immunocompromised patients. Growing antimicrobial resistance (AMR) is a global health concern according to the World Health Organization. Therefore, novel antimicrobial agents, such as plant extracts containing complexes of compounds to which it is impossible to develop resistance, are being investigated. This study aimed to compare the effects of ethyl acetate (EtOAc) extracts from the roots (EtOAcR) and aerial parts (EtOAcAP) of the perennial Bulgarian plant *Geum urbanum* L. (*Rosacea*) on the phenotype inhibition of the Las/RhI quorum sensing (QS) system in *Pseudomonas aeruginosa* PA01 and ATCC 27853, specifically on biofilm formation, swarming motility, pyocyanin production, and gene expression. For this purpose, we used sub-minimal inhibition concentrations (sub-MICs), which do not affect bacterial growth. We found that the evaluated sub-MICs suppressed all studied phenotypic manifestations, with no expression of the target *las*I/*las*R and *rhI*/*rhI*R genes. The observed anti-QS capacity of *G. urbanum* extracts is probably related to their high phenolic content. Moreover, the EtOAcR and EtOAcAP extracts showed effective antioxidant capacity via DPPH, ABTS and superoxide radical scavenging effects, as well copper ion chelation activity. These findings will support the development of novel phytocomplexes applicable for the biocontrol and prevention of *P. aeruginosa* infections.

Geum urbanum L.

Introduction

Biofilm formation occurs in five stages: 1) cell adhesion; 2) reversible adhesion; 3) irreversible adhesion; 4) maturation and 5) cell detachment. At the surface of the biofilm, where oxygen and nutrients are abundant, living, actively dividing bacterial cells can be found. Deeper into the biofilm, slower-growing and dying cells are found. Most antibiotics damage actively dividing cells and therefore they do not destroy bacterial biofilms, leading to the persistence and recurrence of *P. aeruginosa* infections. Biofilm formation is associated with AMR, DNA transfer, carrying out metabolic activities and other processes. These factors are regulated by the expression of genes combined in a system called QS. The bacterial density coordinates the activation of the latter through the induction of specific receptor signaling complexes called autoinducers (AIs). AIs are signaling molecules synthesized during a particular growth phase or in response to specific environmental changes. When the bacterial density increases, their concentration in the environment also increases.



Legend: 3-oxo-C12-HSL – N-3-oxododecanoyl-homoserine lactone, C4-HSL – N-butyryl-homoserine lactone, PQS – *Pseudomonas* quinolone system

The activation of PQS leads to the synthesis of multiple virulence factors, such as pyocyanin and elastase. At a high cell density, the Las system is activated when LasR interacts with 3-oxo-C12-HSL, leading to the synthesis of LasB elastase, LasA protease, Aβr alkaline protease and exotoxin A. RhlR interacts with C4-HSL and lead to increased synthesis of cytotoxic lectins, pyocyanin, HCN, rhamnolipids and swarming motility control. The biofilm formation depends on the synthesis of 3-oxo-C12-HSL and C4-HSL.

Methodology

Extract preparation

- Dry roots (500 g) and aerial parts (500 g) were macerated for 2 days in 3L MeOH. The fractions were concentrated by a vacuum rotary evaporator, followed by sequential extraction with petroleum ether and EtOAc. The extracts were evaporated to dry mass (10.4 g EtOAcAP and and 14.1 g EtOAcR).

Microbiological tests

- Determination of minimum inhibitory concentration (MIC) to choose sub-MICs;
- Determination of biofilm inhibition potential;
- Determination of pyocyanin production quantity;
- Determination of swarming motility;
- PCR Analysis.

Antioxidant activity

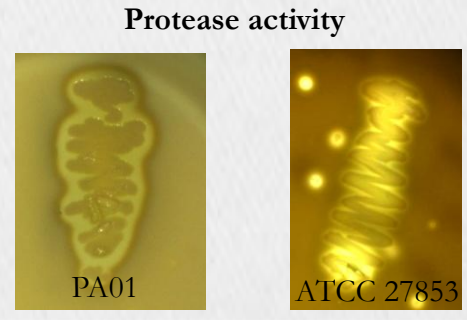
- Determination of the total flavonoids and polyphenol content;
- DPPH radical scavenging assay;
- Superoxide anion radical generating system;
- Ferric-reducing antioxidant power ;
- Iron-chelating power;
- Cupric-reducing antioxidant capacity.

Results

Determination of MIC (mg/mL)

Strain/ extract	*P. aeruginosa*	
	PA01	ATCC 27853
EtOAcAP	6.25	25
EtOAcR	6.25	12.5

Protease activity



Antioxidant capacity of EtOAc extracts

Extracts / Parameters	TPC [µg Galic Acid/mg Extract]	TFC [µg Quercetin /mg Extract]	FRAP IC_{50}	CUPRAC IC_{50}	Fe (II)-Chelating IC_{50}	DPPH Scavenging Activity, IC_{50}, [mg/mL]	Superoxide Scavenging, IC_{50}[mg/mL]	ABTS IC_{50}[mg/mL]
EtOAcAP	5.92 ± 0.23	0.98 ± 0.05	4.8 ± 0.42	16.80± 5.87	1.50 ± 0.03	19.50 ± 1.02	4.95 ± 0.048	31.85 ± 0.26
EtOAcR	0.96 ± 0.08	0.22 ± 0.08	15.20 ± 2.13	33.3 ± 2.85	1.07 ± 0.05	67.4 ± 3.21	17.84 ± 0.15	62.25 ± 0.46

Legend: TPC - total polyphenolic content and TFC – total flavonoid

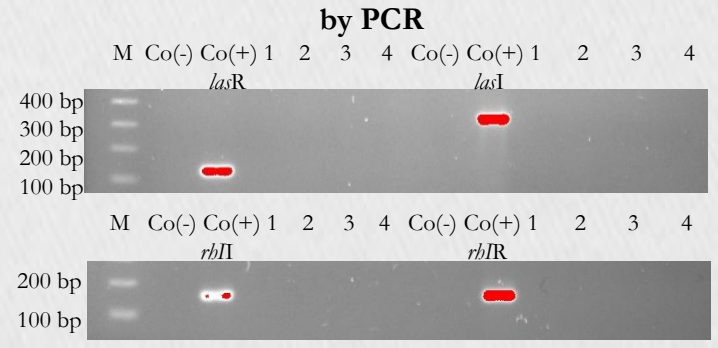
Inhibition of PA01 phenotype

Sub-MICs / extract	3.12 mg/mL	1.56 mg/mL	0.098 mg/mL
Biofilm formation (%)			
EtOAcAP	14.3 ± 0.03	36 ± 0.01	90.6 ± 0.03
EtOAcR	12.7 ±0.04	31.1 ±0.03	66.6 ± 0.05
Control	100 ± 0.05		
Sub-MICs / extract	**2.5 mg/mL**	**1.25 mg/mL**	**0.312 mg/mL**
Amount of pyocyanin (µg/mL)			
EtOAcAP	0.35 ± 0.01	0.51 ± 0.03	2.43 ± 0.02
EtOAcR	0.48 ± 0.05	0.82 ± 0.04	1.2 ± 0.01
Control	5.46 ± 0.02		

Inhibition of *P. aeruginosa* ATCC 27853 phenotype

Sub-MICs / extract	6.25 mg/mL	3.12 mg/mL	1.56 mg/mL
Biofilm formation (%)			
EtOAcAP	38.4 ± 0.02	61.3 ± 0.04	97.1 ± 0.02
EtOAcR	15.1 ± 0.05	16.2 ± 0.03	99.5 ± 0.01
Control	100 ± 0.05		
Amount of pyocyanin (µg/mL)			
EtOAcAP	1.99 ± 0.05	2.12 ± 0.02	2.29 ± 0.04
EtOAcR	1.91 ± 0.03	1.98 ± 0.01	2.02 ± 0.05
Control	2.7 ± 0.02		

Detection of *las*I/*las*R and *rhI*/*rhI*R genes by PCR



Legend: M - marker; Co(−) - negative control; Co(+) - positive control. For *P. aeruginosa* ATCC 27853: 1) EtOAcAP 6.25 mg/mL, 2) EtOAcAP 3.12 mg/mL, 3) EtOAcR 6.25 mg/mL and 4) EtOAc 3.12 mg/mL. For PA01: 1) EtOAcAP 3.12 mg/mL, 2) EtOAcAP 1.56 mg/mL, 3) EtOAcR 3.12 mg/mL and 4) EtOAc 1.56 mg/mL

Inhibition of swarming motility

Conclusions

EtOAc extracts from aerial parts and roots of the herb *Geum urbanum* L. inhibit 100% of the expression of the Las/RhI gene regulatory system, pyocyanin synthesis, bacterial motility, and the biofilm formation by *Pseudomonas aeruginosa* Las/RhI pathogenic factors. Therefore, we assumed that the tested phenotypic expression was significantly influenced by the third QS system, PQS. Furthermore, as free radical scavengers and metal-chelating and metal-reducing agents, both extracts showed strong redox-modulating potential. The current study's findings demonstrate once again how plant extracts with a high content of secondary metabolites can have a multitargeted effect to limit the degree of damage caused by bacterial infections.

References:

1. Dimitrova, L., Popova, M., Bankova, V., & Najdenski, H. (2019). Anti-quorum sensing potential of *Geum urbanum* L. *Comptes Rendus Acad. Bulg. Des Sci*, *72*, 341-349.
2. Dimitrova, L., Mileva, M., Georgieva, A., Tzvetanova, E., Popova, M., Bankova, V., & Najdenski, H. (2025). redox-modulating capacity and effect of ethyl acetate roots and aerial parts extracts from *Geum urbanum* L. on the phenotype inhibition of the *Pseudomonas aeruginosa* Las/RhI quorum sensing system. *Plants*, 14(2), 213.