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Antibiofilm activity of *Juglans regia* L. extracts against waterborne isolates of *Pseudomonas aeruginosa*

Chaired by **Dr. Alfredo Berzal-Herranz** and **Prof. Dr. Maria Emília Sousa**

pharmaceuticals MDPI

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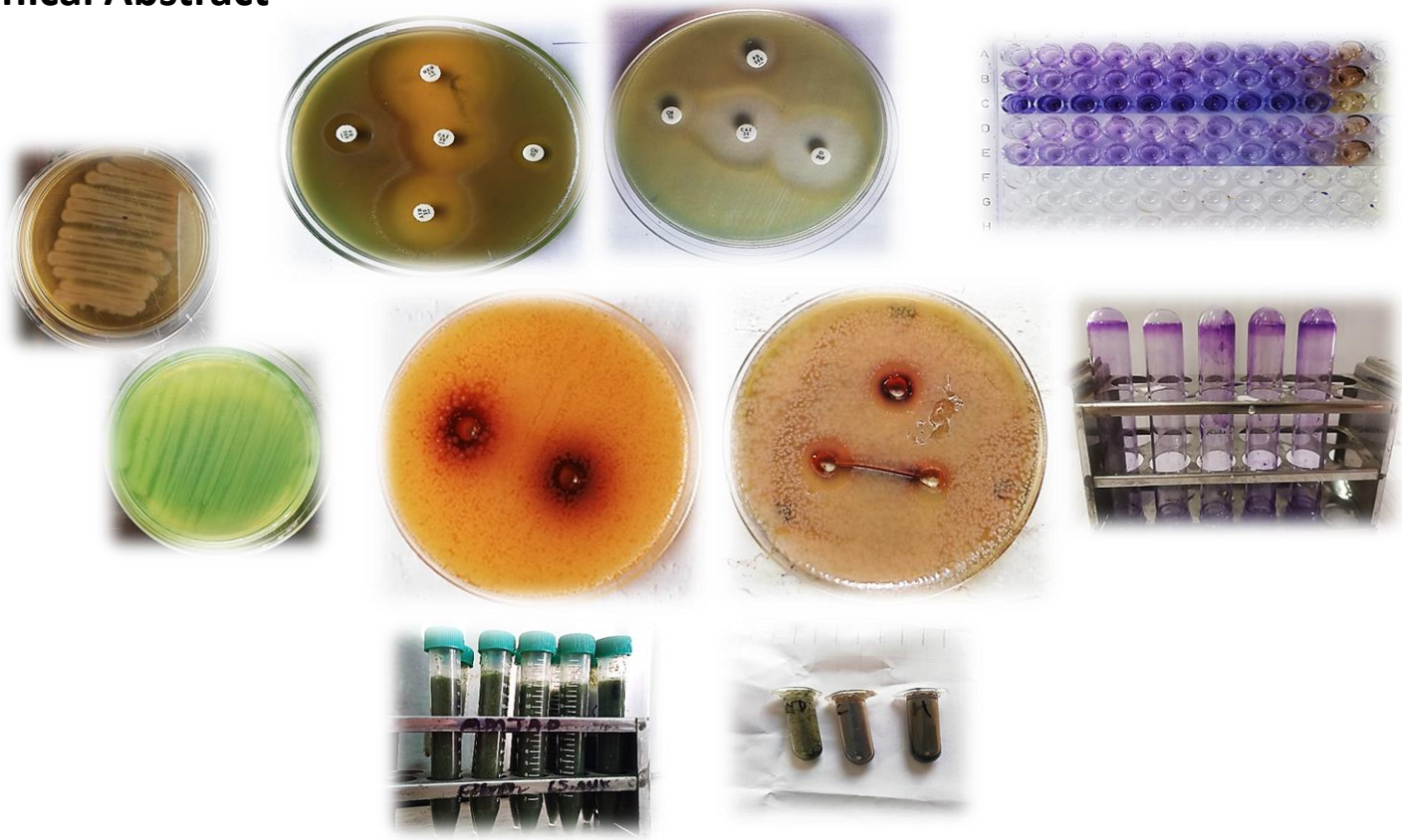
Graphical Abstract



Abstract:

Pseudomonas aeruginosa is an opportunistic and nosocomial pathogen associated with respiratory tract infections. Due to the irrational use of antibiotics, *P. aeruginosa* has developed antibiotic resistance, resulting in increased morbidity and mortality rates. Medicinal plants of reported antibacterial activity can be alternatively used to treat its infections and do not result in resistance. The purpose of our study was to check for the antibiofilm potential of *Juglans regia* L. (*Persian walnut*) extracts against waterborne *P. aeruginosa*. The aqueous and methanol extracts were checked for antibacterial activity by agar well diffusion method. The biofilm formation assay carried out for the plant extracts gave MIC values of 3 and 5 µg/ml for *P. aeruginosa* strains. The thin layer chromatography (TLC), revealed the active components in the form of two bands. Our study indicated that the native plants of Pakistan have bioactivity against biofilm-forming pathogens.

Keywords: Biofilm forming pathogens; *Juglans regia*; Persian walnut

Introduction

- Plants are the traditional source for bioactive compounds
- About 80% of individuals from developed countries use traditional medicines made from such plants [1]
- Medicinal plants are the best source to obtain natural medicines as well as finding new ones [1]

[1] Priya Joy and Dr. D. Patric Raja Anti-Bacterial Activity Studies of *Jasminum grandiflorum* and *Jasminum sambac* Ethnobotanical Leaflets 12: 481-483. 2008.

Introduction (Cont.)

Juglans regia

- Juglandaceae
- Common names: Persian walnut, English walnut, Madeira walnut

Uses

- Ethno veterinary plant
- Diarrhea, arthritis, asthma, skin disorders
- Endocrine diseases such as diabetes mellitus, anorexia

Photo courtesy: Mr. Rashid Nawaz

Bioactivity

- Antibacterial
- Antifungal
- Anti-inflammatory [2]

[2] Bhat AA, Shakeel A, Rafiq S, Farooq I, Malik AQ, Alghuthami ME, Alharthi S, Qanash H, Alharthy SA. 2023. Juglans regia Linn.: A Natural Repository of Vital Phytochemical and Pharmacological Compounds. Life. 13(2): 380.

Results and discussion

- **Sub-culturing of water-borne *P. aeruginosa***

The strains of *P. aeruginosa* were isolated from water samples by membrane filtration method and were cultured on Cetrimide agar. The cultures were characterized through microscopy (Gram staining) and biochemical testing

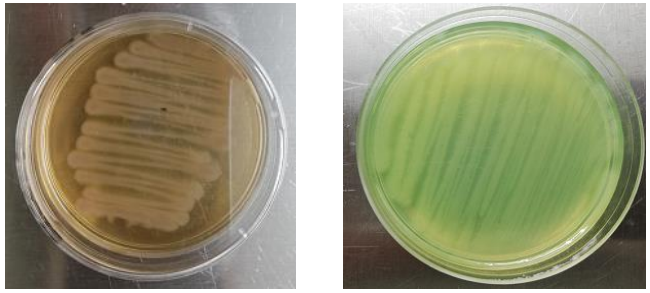


Photo courtesy: Mr. Rashid Nawaz

Results and discussion (Cont.)

- **Antibiotic susceptibility profiling**

The suceptibility and resistance patterns of waterborne *P. aeroginosa* isolates (N= 16) were determined against commonly prescribed antibiotics (CLSI standard 2020)

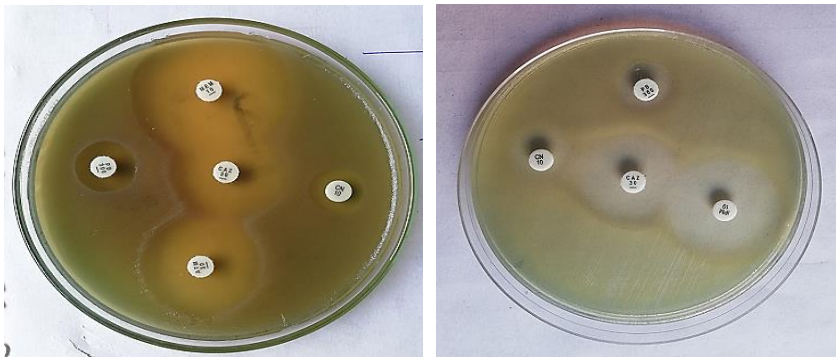


Photo courtesy: Mr. Rashid Nawaz

Results and discussion (Cont.)

CAZ=Ceftazidime, AZM= Aztreonam, MPM= Meropenem, GEN= Gentamycin

P. aeruginosa Isolates	Zone of inhibition in (mm)			
	CAZ	AZM	MPM	GEN
PA - 01	13	14	16	6
PA - 02	13	14	18	6
PA - 03	12	13	17	9
PA - 04	13	14	14	7
PA - 05	13	15	18	9
PA - 06	14	14	15	6
PA - 07	28	26	40	16
PA - 08	29	30	42	17
PA - 09	30	30	44	15
PA - 10	26	27	42	14
PA - 11	13	14	12	6
PA - 12	30	27	41	16
PA - 13	26	31	43	15
PA - 14	12	14	14	7
PA - 15	30	28	40	14
PA - 16	26	26	43	18

Results and discussion (Cont.)

- ***In vitro* antimicrobial activity of *J. regia* extracts against MDR resistant waterborne isolates of *P. aeruginosa***

Extracts of *J. regia* showed bioactivity against MDR *P. aeruginosa* isolates (N=5)

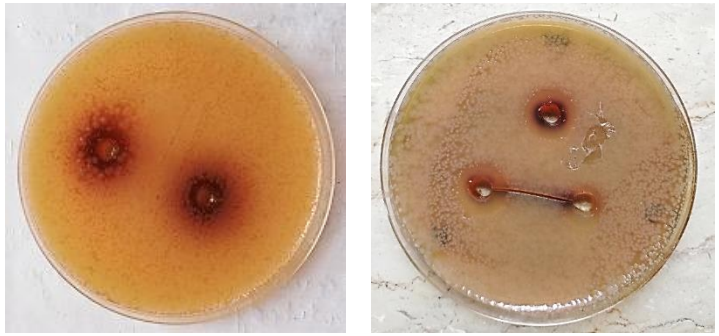


Photo courtesy: Mr. Rashid Nawaz

Results and discussion (Cont.)

Sample		Isolates	Zone of inhibition (mm)	
J. regia	Aqueous (cold)	PA-04	08	09±1.00
			10	
			09	
		PA-06	09	10±1.00
			11	
			10	
		PA-08	10	11±1.00
			12	
			11	
		PA-11	13	12±1.00
			12	
			14	
		PA-14	10	09±1.00
			09	
			08	

Sample		Isolates	Zone of inhibition (mm)	
J. regia	Aqueous (hot)	PA-04	11	12±1.00
			13	
			12	
		PA-06	10	10±1.00
			09	
			11	
		PA-08	11	12±1.00
			13	
			12	
		PA-11	12	11±1.00
			11	
			10	
		PA-14	14	12±1.00
			13	
			12	

Results and discussion (Cont.)

Sample		Isolates	Zone of inhibition (mm)	
J. regia	Methanol	PA-04	13	13 ±1.00
			12	
			14	
		PA-06	12	11 ±1.00
			11	
			10	
		PA-08	13	12 ±1.00
			12	
			11	
		PA-11	10	10 ±1.00
			09	
			11	
		PA-14	11	11 ±1.00
			12	
			10	

Results and discussion (Cont.)

- **Biofilm formation (Tube assay)**

- Using the qualitative analysis with TSB + 2% glucose solution and staining with 0.1% (w/v) crystal violet

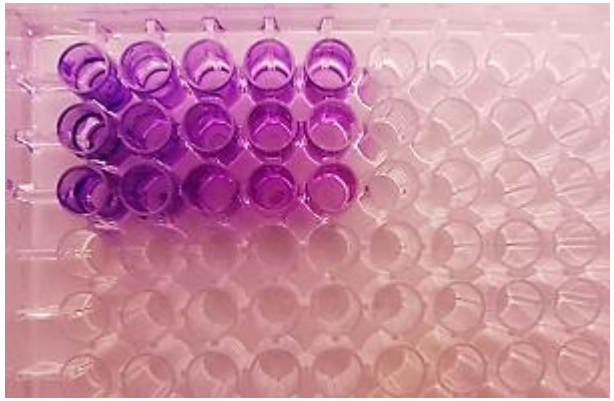
- Five biofilm forming waterborne *P. aeruginosa* strains were observed

Photo courtesy: Mr. Rashid Nawaz

Results and discussion (Cont.)

- **Antibiofilm formation assay**

- Biofilm formation was recorded through ELISA plate reader at 630nm absorbance
- Highest dilution of MDR isolates of *P. aeruginosa* resulted in biofilm formation
- Moderate formation of biofilm observed in the presence of *J. regia* extracts



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Results and discussion (Cont.)

- **Minimum inhibitory concentration (MIC) of *J. regia* extracts against biofilm forming *P. aeruginosa* strains**

The MIC of *J. regia* extracts was determined by broth microdilution method

Serial no.	Plant	Plant Extract		MIC Value	Mean ±SE
1	*J. regia*	Aqueous (cold)	PA - 01	3.90	3.12± 0.47
			PA - 02	3.906	
			PA - 03	1.953	
			PA - 04	3.906	
			PA - 05	1.953	
		Aqueous (hot)	PA - 01	7.182	5.21± 0.80
			PA - 02	3.906	
			PA - 03	7.182	
			PA - 04	3.906	
			PA - 05	3.906	
		Methanol	PA - 01	3.906	5.87± 0.80
			PA - 02	7.182	
			PA - 03	7.182	
			PA - 04	3.906	
			PA - 05	7.182	

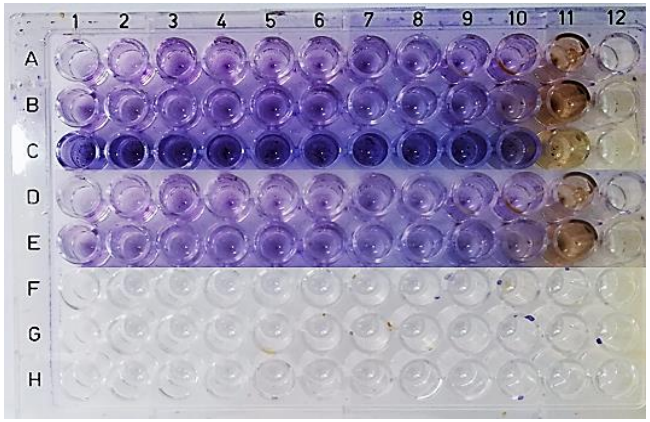

Photo courtesy: Mr. Rashid Nawaz

Conclusions

J. regia leaves extracts

- Previously reported antibacterial and antibiofilm potential against clinical isolates of *P. aeruginosa*

Our study

- *J. regia* extracts possess activity against waterborne isolates of *P. aeruginosa*
- Showed antibiofilm activity

Future exploration

- Screening for quorum sensing genes in these isolates and also for resistance genes against prescribed antibiotics

Acknowledgments

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