Iraqi Journal of Biotechnology, 2025, Vol. 24, Special Issue 2, 593-598

The Impact of *Tecoma stans* ethanolic Extract on Gens Expression of *MexA and MexB* of *Pseudomonas aeruginosa*.

[1]Yahya M. Flieh , [2]M.I. Salih

[1,2] Institute of Genetic Engineering and Biotechnology for Postgraduate Studies, University of Baghdad

Received: February 20, 2025 / Accepted: May 5, 2025 / Published: November 16, 2025

Abstract: An important therapeutic challenge in clinical settings is *Pseudomonas aeruginosa* a common opportunistic bacterium linked to drug resistance. *Pseudomonas aeruginosa* contains MexA and MexB as part of its MexAB-OprM efflux pump. This pump aids in the bacteria natural resistance to a variety of antibiotics. This efflux pump contributes to bacterial resistance to antibiotics. Finding out if the ethanolic 70% extract of *Tecoma stans* altered the expression of the genes encoding these efflux pump proteins was the aim of this study. Regarding clinical isolates of *P. aeruginosa* employing RT-qPCR and MIC to assess the gene expression of the ethanolic extract of the dried leaves of *Tecoma stans*. The results showed that the treated groups levels of MexA and MexB gene expression were significantly lower than those of the untreated control group. This suggests that by blocking the efflux pump *Tecoma stans* extract can increase the effectiveness of drugs against the resistant strain. *Tecoma stan* components may naturally block the mechanisms causing bacterial multidrug resistance according to the study.

Keywords: *P. aeruginosa; MexA, MexB gene expression; Tecoma stan; ethanolic extract*

Corresponding author: (Email: **Yahia.Faleeh2300m@ige.uobaghdad.edu.iq**).

Introduction

Bacterial infections which can cause burn wound sepsis and infection due to changes in skin physiology are the primary cause of complications and deaths among burn patients (1). Patients with a rip in their protective skin barrier are more susceptible to bacterial infection and colonization by pathogenic bacteria like (2). *P. aeruginosa* is a common Gram-negative bacterium that belongs to the Pseudomonadaceae family and can flourish in a range of environments. Numerous organisms including plants animals and—most importantly—humans can become infected by the opportunistic pathogen Pseudomonas aeruginosa. Specifically, patients with burn injuries immunocompromised individuals and those with cystic fibrosis (3). This bacterium is a facultative anaerobe capable of utilizing a wide range of organic and inorganic compounds as carbon sources(4). Because of its multidrug resistance (MDR) phenotype, *P. aeruginosa* poses a serious clinical challenge. Antimicrobial resistance can be caused by a number of mechanisms such as enzyme synthesis protein loss from the outer membrane target changes and multidrug efflux systems. The resistance-nodulation-division (RND) family of drug/proton antiporters in particular the Mex efflux

pumps is an essential part of aeruginosa. Antibiotic resistance is largely caused by the MexAB-OprM system and these pumps serve as essential defenses accelerating the emergence of strains that are extensively drug-resistant (XDR) and pan drug-resistant (PDR). (5).

As antimicrobial resistance increases and conventional antimicrobial medications become less effective, alternative approaches to combating microorganisms are gaining popularity. Plant-based natural compounds known as phytochemicals have drawn interest as potential antimicrobial defense mechanisms. These bioactive substances have demonstrated potential in strengthening the body's defenses against microbial assaults and have a variety of therapeutic uses (6). *Pseudomonas aeruginosais* an opportunistic pathogen with a high level of treatment resistance. because of the outer permeability barrier which it is naturally resistant to manyantibiotics (7). Pseudomonas aeruginosais a virulent agent having a tendency to develop resistance to majority of the antibiotics available for the treatment. It is a leading cause of life-threateningnosocomial infections. It's intrinsic resistance to many antimicrobial agents and development of multidrug resistance imposes severe therapeutic problem for clinicians (8).

Common names for *Tecoma stans* var. stans (L.) Juss. ex Kunth (Bignoniaceae) include kusi urakame, koyawari, Palo amarillo, tronadora, yellow-elder, yellow trumpet bush, trumpet-flower, yellow bells, trumpet bush, ginger-thomas, esperanza, and timboco. It is native to the arid ecosystems of North America and the high altitude regions of South America. (9). This plant, which has naturalized in tropical and subtropical areas of Asia, Oceania, and Africa, has antibacterial, antidiabetic, antiproliferative, anti-inflammatory, and antioxidant qualities (10).

This study aimed to evaluate the effect of a 70% ethanolic extract of Tecoma stans on the gene expression of MexA and MexB in Pseudomonas aeruginosa. It also aimed to determine the extract's minimum inhibitory concentration (MIC) and investigate its potential as a natural efflux pump inhibitor to enhance antibiotic effectiveness against multidrug-resistant strains.

Material and methods

Sample Collection

A total of 100 clinical samples were obtained from burn and wound patients who attended treatment at Nasiriyah General Hospital in Thi Qar Governorate during the period from September 2024 to January 2025.

Isolation and Identification of Pseudomonas aeruginosa

Laboratory diagnosis was made based on biochemical and morphological tests, and the VITEK-2 Compact system was used for confirmation. 36 *P. aeruginos* isolates were identified from a total of 100 samples, and all *P. aeruginosa* isolates were tested for sensitivity to nine types of antibiotics. The results showed that the isolates were resistant to most of the antibiotics used in this study, the results showed that all strains of *P. aeruginosa* highest resistance to Cefazolin, Cefepim, Gentamicin, Amikacin and Ciprofloxacin with a prevalence of (100.0%). and resistance to Ceftazidime (62.86) and Piperacillin/tazobactam at (51.43%) and the lowest resistance was Imipenem. (5.71%) and Meropenem (11.43%), and ten isolates with multiple

antibiotic resistance were selected.

Collection of (*Tecoma stans. L*)

The leaves of *Tecoma stans* were collected from the garden of the internal departments of the Veterinary Medicine college, University of Baghdad, which were identified by the specialist in the Department of Life Sciences, College of Science, University of Baghdad, The leaves were washed with water, dried at room temperature, ground using a mill, and then stored at 4°C for further analysis.

Preparation of *Tecoma stans* Extract

Following air drying in the shade, 100 g of fresh *Tecoma stans* leaves were ground into a powder using a coffee grinder. The powder was placed in a volumetric cylindrical flask with 1000 ml of 70% ethyl alcohol to create a ratio of 1/10 (W/V). Following a 24-hour period of mixing the solution with a magnetic stirrer device, the liquid was filtered through four layers of medical gauze before being filtered once more via Whatman (No. 1) filter paper. To get crude extract, the filtered mixture was concentrated for 72 hours at 40°C in an incubator. The extract, which had a yield of 10 grams, was kept at 4°C in a dark, sterile screw bottle until it was needed (11).

Determination of Minimum Inhibitory Concentration (MIC) of *Tecoma stans*

The minimum inhibitory concentrations (MIC) of *Tecoma stans* extract were determined using a 96-well microtiter plate and broth microdilution method. The extract was prepared by constructing successive two-fold dilutions on the plate. The working solution was made with broth containing 256 mg/ml. The extract was then moved to the next row, mixed with 100 µl of broth, and incubated at 37°C for 18-20 hours. The lowest extract concentrations were visually determined using broth microdilutions (12).

Preparation of primers

Primers that used in this study are listed in (Table 1)

Analysis of (qRT- PCR) Assay

The Qubit® 1st-Step RT-qPCR System (Qubit®- USA) was used to amplify mRNA particles. The amplification reaction was done using the master amplification reaction listed in Table (2), together with the Qubit 2nd-Step RT-PCR and the Qubit®-USA listed in Table (3). Experiments were conducted to synthesize various properties of annealing temperature and..cDNA.

The approach of Delta Ct (ΔΔCt)

Relative gene expression was analyzed using the comparative threshold cycle (Ct) method, also known as the Livak method (13). A calibrator sample—such as an untreated control or any appropriate reference—was used to normalize expression levels across samples.

Statistical analysis

The statistical study was conducted using SPSS version 26, developed by IBM Company in Chicago, Illinois. The molecular result was exploration by using Chi-square ($\chi 2$) test. *P* values less than (0.05) is considered. Data were expressed as mean ± SD.

Table (1): - Primers used in this Study

Primer name		Sequence (5´-3´)	Production size	Reference
MexA	F	CAACAGCTCGACCCGATCTAC	171 bp	Newly Designed
	R	GAATTCGAGGCGACCTTCCAG		
MexB	F	TCCTCGTGTTCCTGGTGATGT	129 bp	
	R	TGTTGATCGAGAAGCCGAACG		
16s rRNA	F	CCGCTAATACCGCATACGTCC	102 bp	
	R	GCCTTTACCCCACCAACTAGC		

Table (2): Components of RT-PCR interaction.

Component	Volume per 20µl Reaction
Luna Universal qPCR Master Mix	10 µl
Forward Primer	1 µl
Reverse Primer	1 µl
cDNA Template	5 µl
Nuclease-free water	3 µl
Total	20µl

Result and discussion

Quantification of MexA and MexB Expression by Real-Time PCR

Amplification was measured as a cycle threshold or Ct value which shows that low Ct values indicate high gene expression and high Ct values indicate low gene expression, after treatment with *Tecoma stanc* ethanolic extract for *p. aeruginosa mexA* and *mexB gene*, as shown in Tables 3, 4. The *Tecoma stans* ethanolic extract effect on gene expression of *P. aeruginosa mexA and mexB gene*.The infamous Gram-negative bacterium *P. aeruginosa* is well-known for its multidrug resistance (MDR), which is mostly caused by its efflux pump systems, especially the *MexAB-OprM* pump, which is controlled by genes like *mexA*, natural substances that can block these efflux pumps have drawn a lot of interest since they may be able to increase the effectiveness of currently used antibiotics (14).

Studies have demonstrated that some flavonoids may inhibit the expression of efflux pump genes, including mexA, in *P. aeruginosa* (15) , additionally, quercetin, a flavonoid found in various plants, has been found to downregulate the expression of mexA and other efflux pump genes, increasing *P. aeruginosa* susceptibility to antibiotics. *Tecoma stans* is a plant that contains a number of biologically active chemicals, including flavonoids, a class of compounds derived from plants known for their wide-spectrum antimicrobial activity (16). Quinones are another compound with potent antibacterial qualities that may be able to reduce *P. aeruginosa* efflux pumps. For example, anthraquinones had been demonstrated to inhibit the activity of the MexAB-OprM efflux pump effectively blocking *P. aeruginosa*. Alkaloids are another class of bioactive compounds found in *Tecoma stans* which make *P. aeruginosa* more vulnerable to antibiotics. Additionally, contain terpenoids, especially triterpenes, which are recognized for their antibacterial qualities. It has been demonstrated that certain triterpenoids prevent *P. aeruginosa* efflux pumps from functioning, also, observed that the triterpenoid ursolic acid, which is present in a variety of plants, inhibits the MexAB-OprM efflux pump, increasing the effectiveness of antibiotics against *P. aeruginosa* (17). The use of *Tecoma stans* extracts may lead to a significant reduction in MexA gene expression, as evidenced by studies utilizing synthetic siRNA targeting this gene, which resulted in decreased mRNA levels (18).

Comparative Analysis: Although Me xA and MexB were not specically studi ed, their relationship implies that since both are a member of the same efflux sy stem, blocking MexA may have an indir ect effect on MexB expression (19). The antibacterial activity of *Tecoma stans* extracts' antibacterial qualities, as shown by a number of tests, imply that they may reduce resistance by lowering the expression of MexA and MexB (20).

Table (3): shows the gene expression of *p. aeruginosa mexA gene* before and after treatment with *Tecoma stans* ehanolic extract.

	Samples	CT *16sRNA*	CT *mexA*	ΔCT	ΔΔCT	Fold	MEAN
Before treatment	**1.**	11.89	13.96	2.07	0	1	1.00 ±0.00 a
	2.	14.56	21.37	6.81	0	1	
	3.	9.16	12.06	2.9	0	1	
	4.	9.78	12.8	3.02	0	1	
	5.	13.44	11.66	-1.78	0	1	
After treatment	**1.**	9.95	13.3	3.35	1.28	0.41	0.2 ± 0.12 b
	2.	9.4	17.19	7.79	0.98	0.50	
	3.	8.56	16.15	7.59	4.69	0.03	
	4.	8.74	16.08	7.34	4.32	0.05	
	5.	9.44	13.47	4.03	5.81	0.01	
**p<0.01							

Table (4): shows the gene expression of *p. aeruginosa mexB gene* before and after treatment with *Tecoma stans* ehanolic extract.

	Samples	CT *16sRNA*	CT *mexb*	ΔCT	ΔΔCT	Fold	MEAN
Befor treatment	1.	11.89	14.03	2.14	0	1	1.00 ±0.00 a
	2.	14.56	15.13	0.57	0	1	
	3.	9.16	12.29	3.13	0	1	
	4.	9.78	13.07	3.29	0	1	
	5.	13.44	12.93	-0.51	0	1	
After treatment	1.	9.95	13.35	3.4	1.26	0.41	0.118 ± 0.076 b
	2.	9.4	16.33	6.93	6.36	0.01	
	3.	8.56	15.55	6.99	3.86	0.06	
	4.	8.74	15.38	6.64	3.35	0.09	
	5.	9.44	14.04	4.6	5.11	0.02	
**p<0.01							

Conclusion

The present research shows that *Tecoma stans'* ethanolic extract drastically reduced the expression of the MexA and MexB genes in *P. aeruginosa* clinical isolates, revealing that it might possess the ability to block the efflux pump. The extract may increase the value of antibiotics against resistant strains of *P. aeruginosa* by reducing the expression of these genes. *Tecoma stans* components may naturally inhibit the mechanisms causing bacterial multidrug resistance, according to the study. These results show *Tecoma stans'* potential as an additional treatment to fight antibiotic resistance, providing an innovative treatment approach for clinical application.

References

1. Darweesh, A. H. K. and Luti, K. J. K. (2024). Utilization of Limosilactobacillus fermentum Cells in a Gel Formula as a Dermal Probiotic against MDR Pseudomonas aeruginosa Associated with

Burn Wound Infection. Iraqi Journal of Science, 6970-6985.
2. AL-Sabagh, F. S. H. and Ghaima, K. K. (2023). Sh. AL-Dabbagh A H. The antibacterial activity of LL-37 peptide against multidrug-resistant Pseudomonas aeruginosa isolated from burn infections. Revista Bionatura, 8(1), 69.
3. Pachori, P.; Gothalwal, R. and Gandhi, P. (2019). Emergence of antibiotic resistance Pseudomonas aeruginosa in intensive care unit; a critical review. Genes and diseases, 6(2), 109-119.
4. Yaseen, A. F., and Al-Draghi, W. A. (2025). Investigate Prevalence of (blaIMP, blaOXA-40, and blaGES) Genes in Carbapenems Resistance Pseudomonas aeruginosa Isolates. Iraqi journal of biotechnology, 24(1).
5. Avakh, A.; Grant, G. D.; Cheesman, M. J.; Kalkundri, T. and Hall, S. (2023). The Art of War with Pseudomonas aeruginosa: Targeting Mex Efflux Pumps Directly to Strategically Enhance Antipseudomonal Drug Efficacy. Antibiotics (Basel, Switzerland), 12(8), 1304.
6. Alanazi, H. H.; Aldughmani, H. A. G. and Mazhari, B. B. Z. (2025). Investigating the Efficacy of Various Natural Products in Raw Form against Multidrug-Resistant Bacteria. Infectious Disorders-Drug Targets, 25(3), E18715265320631. 5234.
7. Obeid, A. A. and Jassim, H. E. (2024) Antibacterial Activity of Chitosan Nanoparticles With Ocimum basilicumoil Extract on Pseudomonas aeruginosa. Iraqi Journal of Biotechnology,23(1)
8. Almusawy, M. N. and Al-Hashimy, B. A. (2022). Role of Some Virulence Genes and Antibiotic Susceptibility of Pseudomonas aeruginosa Isolated from Different Clinical Samples. Iraqi Journal of Biotechnology,21(2)
9. Anand, M. and Basavaraju, R. (2021). A review on phytochemistry and pharmacological uses of Tecoma stans (L.) Juss. ex Kunth. Journal of Ethnopharmacology, 265, 113270.
10. Bakr, R. O.; Fayed, M. A. A.; Salem, M. A. and Hussein, A. S. (2019). Tecoma stans: alkaloid profile and antimicrobial activity. Journal of Pharmacy and Bioallied Sciences, 11(4), 341-347.
11. Jin, J.; Koroleva, O. A.; Gibson, T.; Swanston, J.; Magan, J.; Zhang, Y. A. N., *et al.* (2009). Analysis of phytochemical composition and chemoprotective capacity of rocket (Eruca sativa and Diplotaxis tenuifolia) leafy salad following cultivation in different environments. *Journal of agricultural and food chemistry*, *57*(12), 5227-5234.
12. Ohikhena, F. U.; Wintola, O. A. and Afolayan, A. J. (2017). Evaluation of the Antibacterial and Antifungal Properties of Phragmanthera capitata (Sprengel) Balle (Loranthaceae), a Mistletoe Growing on Rubber Tree, Using the Dilution Techniques. The Scientific World Journal, Volum 2017. Article ID 9658598. 8 pages doi.org/10.1155/2017/9658598.
13. Livak, K. J. and Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCT method. *methods*, *25*(4), 402-408.
14. Kello, E.; Greenberg, R.; Li, W.; Polansky, S.; Maldonado, R.; Peter, Y., et al P. (2023). The Effect of Antibiotic Treatment and Gene Expression of Mex B Efflux Transporters on the Resistance in Pseudomonas Aeruginosa Biofilms. *Applied Microbiology*, *3*(3), 709-721.
15. Waditzer, M. and Bucar, F. (2021). Flavonoids as inhibitors of bacterial efflux pumps. *Molecules*, *26*(22), 6904.
16. Pattnaik, S.; Mishra, M. and Naik, P. K. (2024). Phytochemicals as Potential Antibacterial Agents Against ESKAPE Pathogens. In *ESKAPE Pathogens: Detection, Mechanisms and Treatment Strategies* (pp. 379-419). Singapore: Springer Nature Singapore.
17. Rao, M.; S, P.; Km, D.; Kumar, S.; Nayak, B. B. and Varela, M. F. (2018). *Antimicrobial Compounds of Plant Origin as Efflux Pump Inhibitors: New Avenues for Controlling Multidrug Resistant Pathogens*. *4*(1), 1–6.
18. Al-Qurashi, F. M. S. and Al-Draghi, W. A. (2024). Detection of the effect of synthetic siRNA on efflux pump MexA gene expression and antibiotic resistance in clinical isolates of Pseudomonas aeruginosa. *Biomedicine*, *43*(6),1807–1812.
19. Jasmine, R. and Aishwarya, S. (2012). Role of a novel terpenoid as efflux inhibitor in targeting the efflux protein (mexa) of multi-drug-resistant pseudomonas aeruginosa. *International Journal of Pharmaceutical Sciences and Research*, *3*(6), 1647–1651.
20. Talla, C.; Dongmo Melogmo, Y. K.; Bijou-Lafortune, N. K.; Boyom, F. F., Ngoupayo, J. and Mpondo Mpondo, E. (2024). Unravelling the Antioxidant and Antimicrobial Effect of Tecoma Stans Extracts. *Scholars Academic Journal of Pharmacy*, *13*(09), 317–325.