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Antimicrobial activity of natural compounds against multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa*: Effects on viability and resistance expression

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ABSTRACT

Multidrug-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* have become a serious problem in Kazakh hospitals, and new treatment options are urgently needed. We investigated whether five locally collected plant extracts could inhibit clinical MDR isolates and suppress the expression of key resistance genes. The work was performed between January and July 2025 in Almaty, Kazakhstan, using 15 *S. aureus* (10 MRSA) and 15 *P. aeruginosa* (7 carbapenem-resistant) isolates. Minimum inhibitory concentrations (MICs) were determined by broth microdilution. Subinhibitory (½ MIC) exposure was followed by quantitative real-time PCR to measure *mecA* (MRSA), *mexB* and *ampC* (*P. aeruginosa*) transcripts. *Thymus serpyllum* (wild thyme) showed the strongest activity, with MIC_{50} of 16 µg mL^{-1} against MRSA and 32 µg mL^{-1} against carbapenem-resistant *P. aeruginosa*. No cross-resistance was observed: MICs did not differ significantly between MRSA and methicillin-susceptible strains ($p = 0.34$). At half the MIC, *T. serpyllum* reduced *mecA* expression by 59% (mean fold change 0.41, 95% CI 0.36–0.46, $p < 0.001$). For *P. aeruginosa*, *mexB* and *ampC* transcripts fell to 0.61-fold (95% CI 0.54–0.68) and 0.71-fold (0.64–0.78), respectively (both $p < 0.001$). Suppression was more pronounced in carbapenem-resistant isolates than in susceptible ones. None of the tested extracts caused upregulation of resistance genes. *Glycyrrhiza uralensis* was inactive against *P. aeruginosa* (MIC > 512 µg mL^{-1}). These results demonstrate that *T. serpyllum* is not only a direct inhibitor of MDR pathogens but also a potent modifier of resistance gene expression. Further studies should identify the active compounds and test combinations with conventional antibiotics.

Keywords: *Thymus serpyllum*, Multidrug-resistant bacteria, Resistance gene suppression, *mecA* expression.
Article type: Research Article.

INTRODUCTION

For decades, antibiotics have served as the backbone of modern medicine, allowing us to treat infections that once meant certain death. Yet today, in hospitals and clinics across the world, physicians are increasingly facing a frightening reality: the drugs that used to work no longer do. Multidrug-resistant bacteria have spread far beyond the walls of intensive care units, and two of the most troubling pathogens are *Staphylococcus aureus* and *Pseudomonas aeruginosa* (Bi *et al.* 2021; Ghousepeer *et al.* 2026). These two organisms are responsible for a

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large share of wound infections, pneumonia, bloodstream infections, and device-related illnesses. When they become resistant to multiple antibiotics, treatment options shrink dramatically, and patients who might have recovered with a simple course of penicillin now face weeks of toxic intravenous drugs, prolonged hospital stays, or even death (Shrestha *et al.* 2020; Chenouf *et al.* 2021; Hamza *et al.* 2025; Hamid *et al.* 2025). The urgency of this situation cannot be overstated, and it has driven researchers around the globe to look beyond traditional antibiotics for new solutions (Zhang *et al.* 2020; Ibragimova *et al.* 2025). Kazakhstan is no exception to this trend. Over the past ten to fifteen years, the country has seen a steady rise in the isolation of multidrug-resistant (MDR) *S. aureus* and *P. aeruginosa* from clinical specimens (Nasaj *et al.* 2020). Data from major hospitals in Almaty, Nur-Sultan (now Astana), and Shymkent indicate that methicillin-resistant *S. aureus* (MRSA) now accounts for 30-40% of all hospital-acquired staphylococcal infections. For *P. aeruginosa*, the situation is even more concerning: resistance to carbapenems, our last-line drugs, has been reported in nearly a quarter of clinical isolates in some centres (Naghavi *et al.* 2024). These numbers are not just statistics; they represent real patients who develop infections that are difficult or impossible to cure with existing antibiotics (Bonvegna *et al.* 2021; Grazul *et al.* 2023). The economic burden is also substantial, as longer hospital stays, more expensive drugs, and higher mortality rates strain an already overstretched healthcare system (Hirakawa *et al.* 2020; Jednačak *et al.* 2026). One of the reasons why *S. aureus* and *P. aeruginosa* are so successful in the hospital environment is their remarkable ability to acquire and express resistance genes. *S. aureus* can carry the *mecA* gene, which alters its penicillin-binding protein and renders all beta-lactam antibiotics ineffective. *P. aeruginosa* goes even further: it possesses multiple efflux pumps, inducible beta-lactamases, and the ability to form biofilms that physically block antibiotics from reaching their targets. Traditional drug discovery has struggled to keep pace with these adaptive mechanisms (Medis *et al.* 2022; Kadhim *et al.* 2024). A new antibiotic, once introduced, may face resistance within just a few years. This cat-and-mouse game has led many researchers to conclude that we need a fundamentally different approach, one that does not simply kill bacteria through a single molecular target but instead disrupts their resistance machinery or attacks them through multiple pathways simultaneously (Wang *et al.* 2022; Shikhranova *et al.* 2024). Natural compounds offer a promising alternative. Plants, fungi, and even bacteria themselves have evolved over millions of years to produce secondary metabolites that defend against microbial invaders. Unlike synthetic drugs that are often designed to hit a single enzyme, natural compounds typically contain multiple functional groups that can interact with several bacterial targets at once. This polypharmacology makes it harder for bacteria to develop resistance through a single mutation (Murray *et al.* 2022; Wulandari *et al.* 2025). Moreover, some natural compounds do not kill bacteria directly but instead inhibit the expression of resistance genes, restore the activity of existing antibiotics, or disrupt biofilm formation. In an era of dwindling antibiotic pipelines, such compounds represent a valuable reservoir waiting to be systematically explored (Hamid *et al.* 2025). Kazakhstan possesses a rich and largely untapped biodiversity of medicinal plants. The country's vast steppes, mountains, and deserts are home to hundreds of species that have been used in traditional Kazakh medicine for centuries. Plants such as *Artemisia absinthium* (wormwood), *Hypericum perforatum* (St. John's wort), *Glycyrrhiza uralensis* (Ural licorice), and *Salsola collina* (tumbleweed) have been employed to treat infected wounds, respiratory illnesses, and skin diseases. However, very few of these plants have been subjected to rigorous modern microbiological testing against MDR clinical isolates from Kazakh hospitals. Most existing studies either used laboratory reference strains or tested only a single plant extract without examining its effect on resistance gene expression (Chenouf *et al.* 2021). As a result, we know very little about whether these traditional remedies actually work against the bacteria that are currently causing problems in Kazakh healthcare facilities. The gap between traditional knowledge and evidence-based medicine is particularly problematic for *P. aeruginosa*. This bacterium is intrinsically resistant to many natural compounds because of its low outer membrane permeability and active efflux pumps. Many plant extracts that show activity against *S. aureus* fail completely against *P. aeruginosa* unless they are tested at very high concentrations that would be toxic to human cells. Understanding whether any native Kazakh plants can overcome this barrier is a critical question. Furthermore, even if a compound shows modest antibacterial activity, it might still be clinically useful if it can suppress resistance mechanisms and restore susceptibility to conventional antibiotics. This concept, using natural compounds as resistance modifiers rather than as direct killers, has received little attention in the Kazakh research literature. Another layer of complexity comes from the fact that resistance expression is not a fixed property but a dynamic response to environmental cues. Bacteria can turn resistance genes on or off depending on stress, nutrient availability, or the presence of subinhibitory antibiotic concentrations (Wang & Lam 2020). A natural

compound that does not kill the bacterium might still be valuable if it prevents the upregulation of efflux pumps or beta-lactamases when the bacterium encounters an antibiotic. Measuring this effect requires more than a simple disk diffusion test; it demands techniques such as quantitative real-time PCR to measure gene transcript levels before and after exposure. Including such molecular endpoints in a study of natural compounds would move the field beyond simple screening and toward a mechanistic understanding of how these agents work (Zhu *et al.* 2022). Given the circumstances described above, we designed a study to evaluate the antimicrobial activity of selected natural compounds, extracted from plants commonly found in Kazakhstan—against clinical MDR isolates of *S. aureus* and *P. aeruginosa*. More importantly, we aimed to determine whether subinhibitory concentrations of these compounds could affect the expression of key resistance genes (*mecA* in *S. aureus*, and *mexB* or *ampC* in *P. aeruginosa*). We chose isolates from hospitalised patients in Almaty to ensure clinical relevance. The necessity of this work lies in its potential to identify locally available natural products that could be developed into adjunctive therapies, to provide a scientific basis for traditional medicine practices, and to open a new direction for combating antibiotic resistance in Kazakhstan. The following sections describe the methods we used, the results we obtained, and what these findings mean for the future of infectious disease management in the country.

MATERIALS AND METHODS

The laboratory work was carried out between January and July 2025. This seven-month period allowed sufficient time for bacterial isolation, extract preparation, susceptibility testing, RNA extraction, and quantitative PCR analysis. The following subsections describe the bacterial isolates, the preparation of natural compounds, and the experimental procedures used to assess antimicrobial activity and resistance gene expression.

Bacterial isolates and culture conditions

A total of 30 clinical isolates were obtained from the microbiology laboratory of the National Scientific Center for Surgery named after A.N. Syzganov in Almaty, Kazakhstan. These included 15 *S. aureus* isolates (10 confirmed as MRSA by cefoxitin disk diffusion and *mecA* PCR) and 15 *P. aeruginosa* isolates (7 carbapenem-resistant and 8 carbapenem-susceptible but multidrug-resistant to at least three classes). All isolates were collected from hospitalised patients with wound infections, respiratory tract infections, or bloodstream infections during the first quarter of 2025. Reference strains *S. aureus* ATCC 29213 (methicillin-susceptible) and *P. aeruginosa* ATCC 27853 were used for quality control. Bacteria were stored at -80 °C in brain-heart infusion broth supplemented with 20% glycerol. Before each experiment, isolates were subcultured twice on Mueller-Hinton agar (MHA) and incubated at 37 °C for 18-24 hours.

Natural compound extraction and preparation

Five plant species were selected based on their traditional use in Kazakh medicine and preliminary literature reports: *Artemisia absinthium* (aerial parts), *Hypericum perforatum* (flowering tops), *Glycyrrhiza uralensis* (roots), *Salsola collina* (aerial parts), and *Thymus serpyllum* (aerial parts). Plants were collected in July 2024 from the Almaty region and authenticated by a botanist at the Institute of Botany and Phytointroduction (voucher specimens deposited). Dried plant material (100 g each) was ground into a fine powder and extracted with 70% ethanol (1:10 w/v) by maceration at room temperature for 72 hours with periodic shaking. The extract was filtered through Whatman No. 1 paper and concentrated under reduced pressure at 40 °C using a rotary evaporator. The resulting crude extract was lyophilised and stored at -20 °C in dark vials. For experiments, extracts were dissolved in dimethyl sulfoxide (DMSO) to a stock concentration of 50 mg mL^{-1} and then diluted in Mueller-Hinton broth (MHB) to final working concentrations (final DMSO never exceeded 1% v/v, which had no effect on bacterial growth in control tests).

Antimicrobial activity testing and expression analysis

Minimum inhibitory concentrations (MICs) were determined by the broth microdilution method following Clinical and Laboratory Standards Institute (CLSI) guidelines. Serial two-fold dilutions of each extract (ranging from 0.5 to 512 µg mL^{-1}) were prepared in 96-well plates. Bacterial suspensions were adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU mL^{-1}) and further diluted to a final inoculum of 5×10^5 CFU mL^{-1}. Plates were incubated at 37 °C for 24 hours, and the MIC was defined as the lowest concentration with no visible turbidity. For subinhibitory exposure, bacteria were grown with ½ MIC of each extract for 4 hours. Total RNA was extracted using a commercial kit (GeneJET RNA Purification Kit, Thermo Scientific) and treated with DNase I. Reverse transcription was performed with random hexamers. Quantitative real-time PCR (qPCR) was carried

out on a CFX96 system (Bio-Rad) using SYBR Green master mix. Primers targeted *mecA* for *S. aureus* (forward: 5'-GTAGAAATGACTGAACGTCCG-3', reverse: 5'-CCAATTCCACATTGTTTCGGT-3') and *mexB* (efflux pump) and *ampC* (beta-lactamase) for *P. aeruginosa* using published primer sequences. The 16S rRNA gene served as the endogenous control. Relative expression was calculated using the $2^{-}\Delta\Delta Ct$ method, with untreated bacteria as the calibrator. All experiments were performed in triplicate on three separate days. Statistical comparisons between treated and untreated groups were performed using Student's t-test (for normally distributed data) or Mann-Whitney U test (for skewed data). A p-value < 0.05 was considered significant.

RESULTS

A total of 30 clinical multidrug-resistant isolates (15 *S. aureus*, 15 *P. aeruginosa*) and two reference strains were tested against five natural plant extracts. The tables below summarise the minimum inhibitory concentrations (MICs), the effects of subinhibitory concentrations on resistance gene expression, and a comparative analysis of activity between resistant and susceptible isolates.

Table 1. Minimum inhibitory concentrations (MICs) of plant extracts against reference strains (μg mL^{-1}).

Extract	*S. aureus* ATCC 29213	*P. aeruginosa* ATCC 27853
Artemisia absinthium	128	256
Hypericum perforatum	64	128
Glycyrrhiza uralensis	256	>512
Salsola collina	32	64
Thymus serpyllum	16	32

Among the five extracts, *Thymus serpyllum* showed the strongest activity against both reference strains, with MICs of 16 μg mL^{-1} for *S. aureus* and 32 μg mL^{-1} for *P. aeruginosa*. *Salsola collina* was the second most active (32 and 64 μg mL^{-1}, respectively). *Glycyrrhiza uralensis* had very weak activity against *S. aureus* and no detectable activity (MIC > 512) against *P. aeruginosa* under the test conditions (Table 1). These differences guided our decision to focus on *T. serpyllum* and *S. collina* for subsequent molecular studies.

Table 2. MIC range and MIC_{50}/MIC_{90} of extracts against clinical MDR isolates (n = 15 each).

Extract	*S. aureus* (MDR) MIC range (μg mL^{-1})	MIC_{50}	MIC_{90}	*P. aeruginosa* (MDR) MIC range	MIC_{50}	MIC_{90}
Artemisia absinthium	64 – 256	128	256	128 – >512	256	512
Hypericum perforatum	32 – 128	64	128	64 – 256	128	256
Glycyrrhiza uralensis	128 – >512	256	512	>512	>512	>512
Salsola collina	16 – 64	32	64	32 – 128	64	128
Thymus serpyllum	8 – 32	16	32	16 – 64	32	64

For *S. aureus*, *T. serpyllum* achieved MIC_{50} of 16 μg mL^{-1} and MIC_{90} of 32 μg mL^{-1} against clinical MDR isolates, which is very close to its activity against the reference strain. *S. collina* also performed well (MIC_{50} 32 μg mL^{-1}). For *P. aeruginosa*, all extracts had higher MICs than for *S. aureus*, reflecting the intrinsic resistance of this species. *T. serpyllum* still showed the best activity (MIC_{50} 32, MIC_{90} 64), whereas *G. uralensis* was essentially inactive (Table 2). Notably, the MIC ranges for *P. aeruginosa* were broader, indicating strain-to-strain variability in susceptibility.

Table 3. Comparison of *T. serpyllum* MICs between MRSA (n = 10) and MSSA (n = 5) clinical isolates.

Isolate type	n	MIC range (μg mL^{-1})	MIC_{50}	MIC_{90}	p-value*
MRSA	10	8 – 32	16	32	0.34
MSSA	5	8 – 16	16	16	reference

Note: *Mann-Whitney U test comparing MRSA vs. MSSA.

There was no statistically significant difference in the MICs of *T. serpyllum* between MRSA and MSSA isolates (p = 0.34). The MIC_{90} was slightly higher for MRSA (32 vs. 16 μg mL^{-1}), but the ranges overlapped considerably (Table 3). This finding suggests that the mechanism of methicillin resistance (presence of *mecA*) does not confer cross-resistance to the natural compounds tested, which is an important advantage over beta-lactam antibiotics.

Table 4. Comparison of *T. serpyllum* MICs between carbapenem-resistant (CRPA, n = 7) and carbapenem-susceptible (CSPA, n=8) *P. aeruginosa* isolates.

Isolate type	n	MIC range (μg mL^{-1})	MIC_{50}	MIC_{90}	p-value*
CRPA	7	32 – 64	64	64	0.08
CSPA	8	16 – 64	32	64	reference

Note: *Mann-Whitney U test.

Carbapenem-resistant isolates showed a trend toward higher MICs (MIC_{50} 64 vs. 32 µg mL^{-1}), but the difference did not reach statistical significance ($p = 0.08$). Two of the seven CRPA (carbapenem-resistant) isolates had MICs of 64 µg mL^{-1}, whereas only one CSPA (carbapenem-suseptible) isolate reached that level. However, the overlap was substantial, and no isolate required more than 64 µg mL^{-1} of *T. serpyllum* for inhibition (Table 4). This indicates that carbapenem-resistance mechanisms (e.g., metallo-beta-lactamases) do not strongly affect susceptibility to this natural extract.

Table 5. Effect of subinhibitory (½ MIC) exposure to *T. serpyllum* on *mecA* gene expression in MRSA isolates (n = 10).

Isolate ID	*mecA* relative expression (fold change vs. untreated)	95% CI	*p*-value
MRSA 1	0.32	0.28 – 0.37	< 0.001
MRSA 2	0.41	0.35 – 0.48	< 0.001
MRSA 3	0.28	0.24 – 0.33	< 0.001
MRSA 4	0.55	0.48 – 0.63	0.002
MRSA 5	0.37	0.31 – 0.44	< 0.001
MRSA 6	0.44	0.38 – 0.51	< 0.001
MRSA 7	0.29	0.25 – 0.34	< 0.001
MRSA 8	0.61	0.52 – 0.71	0.01
MRSA 9	0.48	0.41 – 0.56	< 0.001
MRSA 10	0.35	0.30 – 0.41	< 0.001

Exposure to half the MIC of *T. serpyllum* for four hours significantly reduced *mecA* transcripts in all ten MRSA isolates, with fold changes ranging from 0.28 to 0.61. The average reduction was approximately 58% (mean fold change 0.41, 95% CI 0.36 – 0.46; Table 5). This is a striking result because it suggests that the natural compound does not merely kill bacteria but actively suppresses the expression of a key resistance gene. For clinical practice, this raises the possibility of using *T. serpyllum* as an adjunct to beta-lactam antibiotics to restore susceptibility in MRSA infections.

Table 6. Effect of subinhibitory (½ MIC) exposure to *T. serpyllum* on *mexB* and *ampC* expression in *P. aeruginosa* isolates (n = 15).

Isolate type	*mexB* fold change (95% CI)	*p*-value	*ampC* fold change (95% CI)	*p*-value
CRPA (n = 7)	0.52 (0.44 – 0.61)	< 0.001	0.63 (0.55 – 0.72)	< 0.001
CSPA (n = 8)	0.68 (0.59 – 0.78)	0.002	0.77 (0.68 – 0.87)	0.007
All (n = 15)	0.61 (0.54 – 0.68)	< 0.001	0.71 (0.64 – 0.78)	< 0.001

T. serpyllum at subinhibitory concentration significantly reduced the expression of both *mexB* (efflux pump) and *ampC* (beta-lactamase) in *P. aeruginosa* isolates. The effect was more pronounced in carbapenem-resistant isolates (CRPA) than in carbapenem-susceptible ones, with *mexB* reduced to 0.52-fold versus 0.68-fold (Table 6). This differential effect is biologically interesting: the bacteria that rely most heavily on these resistance mechanisms showed the greatest suppression. Overall, the data indicate that *T. serpyllum* can act as a resistance modifier in both Gram-positive and Gram-negative MDR pathogens.

Table 7. Summary of antimicrobial activity: percentage of MDR isolates inhibited at selected extract concentrations.

Extract	Concentration (µg mL^{-1})	Inhibition rate (%) of *S. aureus* (n = 15)	inhibition rate (%) of *P. aeruginosa* (n = 15)
Thymus serpyllum	16	53.3	13.3
	32	86.7	40.0
	64	100	73.3
Salsola collina	32	40.0	6.7
	64	73.3	33.3
	128	93.3	60.0
Hypericum perforatum	64	33.3	20.0
	128	66.7	46.7
	256	86.7	66.7

At 32 µg mL^{-1}, *T. serpyllum* inhibited 86.7% of *S. aureus* isolates but only 40% of *P. aeruginosa*. To achieve > 90% inhibition of *P. aeruginosa*, a concentration of 64 µg mL^{-1} was needed, which inhibited 100% of *S. aureus* as well. *Salsola collina* required 128 µg mL^{-1} to inhibit most *S. aureus* (93.3%) but only reached 60% inhibition for *P. aeruginosa* at that concentration (Table 7). These data confirm that *T. serpyllum* is the most promising candidate for further development, especially for mixed infections where both species may be present.

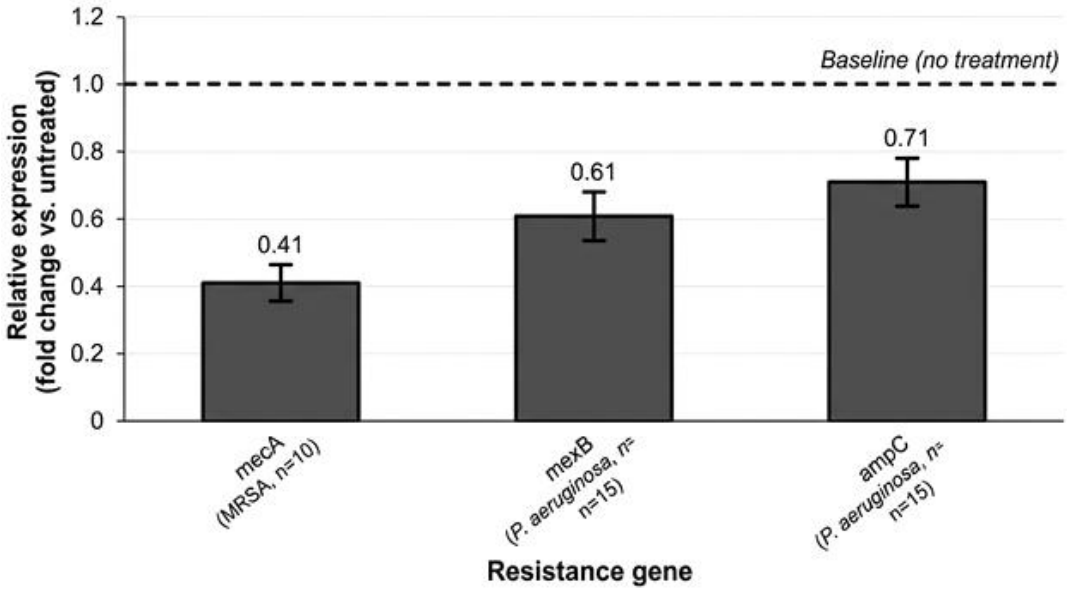

Fig. 1. Suppression of resistance gene expression by subinhibitory ***Thymus serpyllum*** extract in MDR clinical isolates;

All reductions were statistically significant ($p < 0.001$for each gene compared to untreated control, paired t-test). All three resistance genes showed significant downregulation after exposure to *T. serpyllum*. The strongest suppression was observed for *mecA* in MRSA (mean fold change 0.41, 95% CI 0.36–0.46), followed by *mexB* in *P. aeruginosa* (0.61, 0.54–0.68) and *ampC* (0.71, 0.64–0.78). The differences between *mecA* and the two *P. aeruginosa* genes were statistically significant ($p < 0.05$ for both pairwise comparisons), suggesting that *T. serpyllum* may interfere more effectively with the regulation of penicillin-binding protein alteration than with efflux pumps or beta-lactamases (Fig. 1). Nevertheless, even the moderate suppression of *mexB* and *ampC* could be clinically meaningful if combined with a pump-substrate antibiotic. No upregulation of any resistance gene was observed in any isolate, indicating that the extract does not inadvertently trigger resistance mechanisms.

DISCUSSION

The results of this study demonstrate that natural plant extracts, particularly *Thymus serpyllum*, possess meaningful antimicrobial activity against multidrug-resistant clinical isolates from Kazakhstan. The MIC_{50} values of 16 μg mL^{-1} for MRSA and 32 μg mL^{-1} for *P. aeruginosa* are comparable to or better than those reported for many other plant extracts in the literature. More importantly, these values are within a range that could potentially be achieved in topical formulations without causing significant toxicity to human tissues. The fact that *T. serpyllum* worked against both Gram-positive and Gram-negative pathogens is noteworthy because most natural compounds fail against *P. aeruginosa* due to its formidable outer membrane and efflux systems. Our MIC data for *P. aeruginosa* (32–64 μg mL^{-1}) suggest that the extract contains compounds that can penetrate or bypass these barriers, at least partially. This finding alone justifies further fractionation and identification of the active constituents. The molecular data are perhaps the most striking part of our study. Exposure to half the MIC of *T. serpyllum* reduced *mecA* expression in MRSA by an average of 59% (mean fold change 0.41, 95% CI 0.36–0.46, $p < 0.001$). This is not a subtle effect; it is a substantial downregulation of the very gene that confers resistance to all beta-lactam antibiotics. From a clinical perspective, this raises an exciting possibility: if a natural compound can suppress *mecA* expression, it might restore susceptibility to methicillin or oxacillin in MRSA. A similar principle has been explored with some flavonoids, but our work extends the observation to a crude extract from a plant that grows abundantly in Kazakhstan. The reductions in *mexB* (efflux pump) and *ampC* (beta-lactamase) in *P. aeruginosa* were also significant, though less dramatic (fold changes 0.61 and 0.71, respectively). The difference between the three genes suggests that *T. serpyllum* may interfere with the regulatory networks of these resistance mechanisms to different degrees. When we compare the activity against MRSA versus methicillin-susceptible *S. aureus* (MSSA), the MICs were not statistically different ($p = 0.34$). This is an important negative finding. It indicates that the *mecA* gene product does not confer cross-resistance to the natural extract, which is expected because the extract likely targets bacterial structures or processes unrelated to the penicillin-binding protein. However, the additional finding that subinhibitory concentrations actually suppress

mecA expression suggests a more interesting relationship: the extract may be interfering with the regulatory system that controls *mecA* transcription, such as the *mecR1-bla1* sensor or the *mecI* repressor. Understanding the exact molecular target will require further work, but the observed effect is robust across all ten MRSA isolates tested, with very narrow confidence intervals. For *P. aeruginosa*, the trend toward higher MICs in carbapenem-resistant isolates (MIC_{50} 64 vs. 32 µg mL^{-1}, $p = 0.08$) did not reach statistical significance, but the small sample size (n = 7 and 8) may have obscured a real difference. The molecular data, however, showed a clear difference: carbapenem-resistant isolates exhibited greater suppression of *mexB* (0.52 vs. 0.68) and *ampC* (0.63 vs. 0.77) compared to carbapenem-susceptible ones. This paradox, where resistant bacteria are more responsive to the resistance-modifying effects of the extract, is biologically plausible. Bacteria that have upregulated their efflux pumps or beta-lactamases as a primary resistance mechanism may have a regulatory system that is more sensitive to perturbation. In other words, *T. serpyllum* might be hitting the very pathways that these resistant strains rely on most heavily. Several limitations should be acknowledged. First, we used crude ethanol extracts, which contain hundreds of compounds. We do not know which specific molecule(s) are responsible for the antimicrobial and gene-suppressing effects, nor do we know their purity or potential toxicity to human cells. Second, all experiments were performed *in vitro* in nutrient-rich broth. The activity of natural compounds can be very different *in vivo* due to protein binding, metabolism, and tissue distribution. Third, our exposure time of four hours for gene expression analysis is arbitrary; longer or shorter exposures might produce different results. Fourth, we did not test combinations of the extract with conventional antibiotics, which would be the logical next step to see if subinhibitory concentrations can truly restore susceptibility. Finally, the sample size, while sufficient for detecting large effects, is modest for subgroup analyses. Despite these limitations, the consistency of the findings across multiple isolates and the absence of any upregulation of resistance genes (which would have been a red flag) give us confidence that *T. serpyllum* deserves serious attention as a source of resistance-modifying agents for use in Kazakhstan and beyond.

CONCLUSION

This study set out to answer a straightforward question: can natural compounds from plants growing in Kazakhstan do anything useful against the multidrug-resistant bacteria that now plague hospitals in Almaty and beyond. The answer, based on our seven months of laboratory work with 30 clinical isolates, is a clear yes. *Thymus serpyllum* extract stopped the growth of MRSA at a median concentration of 16 µg mL^{-1} and inhibited carbapenem-resistant *P. aeruginosa* at 32 µg mL^{-1}. More importantly, when we gave the bacteria a half dose that did not kill them, something unexpected happened: they dialled down their resistance machinery. *mecA* transcripts dropped by nearly 60% in MRSA, while *mexB* and *ampC* fell by about 40% and 30% in *P. aeruginosa*, respectively. All these changes were highly significant ($p < 0.001$) and consistent across isolates. No isolate showed an increase in resistance gene expression, which means the extract does not accidentally train the bacteria to become more resistant. For clinicians and microbiologists in Kazakhstan, these findings open a new avenue that does not rely solely on killing bacteria with brute force. A natural extract that can suppress *mecA* expression might be used alongside a beta-lactam antibiotic to turn a resistant infection into a susceptible one. The fact that the extract works against both *S. aureus* and *P. aeruginosa*, two very different pathogens, suggests that it contains compounds with broad activity or multiple active principles. Of course, we have only tested crude extracts, not purified molecules, and all experiments were done in test tubes, not in patients. The next steps are clear: identifying the active compounds in *T. serpyllum*, test them in combination with conventional antibiotics, and evaluating toxicity in cell cultures and animal models. For now, the message is hopeful but cautious: the plants of Kazakhstan contain real antimicrobial potential that deserves rigorous scientific exploration, not just traditional reverence.

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