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ORIGINAL ARTICLE

Effects of natural compounds and commercial antibiotics on *Pseudomonas aeruginosa* quorum sensing

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Abstract

Pseudomonas aeruginosa is a Gram-negative bacterium designated by the WHO as a critical priority microorganism due to its virulence, controlled by a quorum sensing (QS) system. QS is regulated through specific subsystems: LasI/LasR, RhlI/RhlR, and PQS/MvfR. Several natural compounds can inhibit these QS mechanisms. In this study, we determined the effect of curcumin, reserpine, and their mixtures with two commercial antibiotics (gentamicin and azithromycin) on *P. aeruginosa* QS mechanisms: *mvfR* gene expression and the production of pyocyanin and rhamnolipids. Antibiotic and natural compounds' minimal inhibitory concentrations (MICs) were determined via microdilution assays. Gentamicin, azithromycin, curcumin, reserpine, and their mixtures exerted variable effects on *mvfR* gene expression, as assessed via semi-quantitative RT-PCR assays. Curcumin, reserpine, and gentamicin inhibited *mvfR* gene expression better than azithromycin, and the mixtures curcumin-gentamicin and reserpine-gentamicin outperformed gentamicin alone in inhibiting *mvfR* gene expression and decreasing pyocyanin and rhamnolipids production, revealing the synergistic effect of these mixture components. The mixtures of curcumin and gentamicin and reserpine and gentamicin may become alternatives to complement or enhance conventional methods currently used to treat *P. aeruginosa* infections.

Keywords: curcumin; gene expression; *Pseudomonas aeruginosa*; quorum sensing; reserpine, virulence factors.

1. Introduction

Pseudomonas aeruginosa is an aerobic, Gram-negative, and mobile bacterium, pathogenic to humans and other animals. *P. aeruginosa* inhibits some cell functions when releasing lipopolysaccharides necessary for infection, and it triggers diseases such as pneumonia, septic shock, and urinary and gastrointestinal tract infections, among others (Bédard *et al.*, 2016; Mielko *et al.*, 2019).

The systematic expression of genes controlling *P. aeruginosa* pathogenicity is population density-related and is known as Quorum Sensing (QS). QS is also involved in the onset of antibiotic resistance phenotypes, biofilm formation, and host immune response (Azam and Khan, 2019; Feng *et al.*, 2016). QS is a communication and signaling system that activates and controls the expression of target genes related to population density sensing and drives the activation of pathogenicity and virulence factors. Three mechanisms (LasI/LasR, RhlI/RhlR, and MvfR/PQS) regulate QS, managing independent gene expression pathways (Laborda *et al.*, 2021; Lee and Zhang, 2015).

The LasI/lasR system leads the expression of genes associated with multiple virulence factors, such as biofilms, motility, and exotoxin synthesis, which are bacterial pathogenesis components. Also, this system is associated with P. aeruginosa bioluminescence (Thorn *et al.*, 2007). The RhlI/RhlR system is responsible for the expression of rhamnolipid and protein expression necessary to reach the host cell cytoplasm. Finally, the PQS system regulates the formation of rhamnolipids and biofilm molecules (Kariminik *et al.*, 2017).

The PQS system underlies *P. aeruginosa* virulence, and the proper functioning of its virulence obeys QS and the synthesis of 4-hydroxy-2-alkylquinolines (HAQ), including the quinolone signal in the system in *P. aeruginosa.* The contribution of the PQS system to P. aeruginosa virulence comes via the transcriptional control of genes outside the RhlI/RhlR regulation system. Also, it enhances the expression of a gene subset that depends on the LasI/LasR and RhlI/RhlR systems, enhancing the virulent action of *P. aeruginosa* (Déziel *et al.*, 2005).

Currently, alternative therapeutic approaches are available. These involve natural products, mainly phytochemicals and their derivatives. Such products have been the most studied due to their antimicrobial properties (Abreu *et al.*, 2012). Furthermore, these phytochemicals have low toxicity, exhibit biochemical specificity, and feature diverse chemical groups. These aspects grant an advantage over conventional antibiotics that gradually lose effectiveness against pathogens (Koehn and Carter, 2005).

Alkaloids are structurally diverse and constitute the active ingredients of many antibacterial medicines. For instance, reserpine, derived from the flowering plant *Rauwolfia serpentine*, is an indole-alkaloid with studied antimalarial, antidepressant, antitumor, and antihypertensive properties (Abdelfatah and Efferth, 2015; Reeta *et al.*, 2013). Also, some reports have presented the reserpine as a flow pump inhibitor and antibacterial (Gibbons, 2004).

Curcumin is another natural compound, known as diferuloylmethane. Obtained from the rhizome of *Turmeric longa* (Naz *et al.*, 2016), curcumin has anti-inflammatory, antifungal, antibacterial, antioxidant, antiviral, and anticancer properties. It has the potential to treat Alzheimer, allergies, diabetes, arthritis, and other chronic diseases. It drives its effects via gene regulation of various growth factors, transcription factors, protein kinase, inflammatory cytokines, and other enzymes (Tyagi *et al.*, 2015).

The antibacterial activity of curcumin in combination with antibiotics may control *P. aeruginosa* infection. Previously published studies addressed curcumin and reserpine biological activities, assessing these natural products with different antimicrobials on *P. aeruginosa* LasI/LasR and RhlI/RhlR QS systems, revealing inhibitory effects on these subsystems and on the virulence factors they regulate (Bahari *et al.*, 2017). However, the scarcity of inhibitory effect studies of these natural compounds individually and mixed with antibiotics on the MvfR/PQS system calls for a deepening of this knowledge and finding synergies between natural and antimicrobial compounds with potential inhibitory effects on QS systems. Especially on the MvfR/PQS subsystem due to its direct regulation of the RhlI/RhlR subsystem, which modulates the production of rhamnolipids and pyocyanin. This work aimed to establish the expression of the *mvfR* gene associated with QS in *P. aeruginosa* after the treatment with reserpine, curcumin, gentamicin, azithromycin and assessing pyocyanin and rhamnolipids virulence factors.

2. Materials and methods

2.1. Bacterial strain and culture conditions

P. aeruginosa ATCC (BAA-47) was reactivated and grown in Luria Bertani broth/agar at 37 °C. Liquid cultures were kept under constant agitation at 200 rpm, and positive growth controls were grown in LB broth with 0.5 % (V/V) of Tween 80 (Inouye *et al.*, 2001). The employed *P. aeruginosa* strain was preserved in LB broth with 20 % glycerol (V/V) at −70 °C.

2.2. Reagents

Gentamicin, azithromycin, reserpine (Sigma Aldrich, USA), and curcumin (Merck, Germany) stock solutions were prepared following CLSI protocols (Cockerill *et al.*, 2013). Stock concentrations were 25 mg ml^{-1} for gentamicin and azithromycin, and 12.5 mg ml^{-1}, in pure DMSO (Sigma Aldrich, USA), for curcumin and reserpine.

2.3. Minimum inhibitory concentrations

Each compound's minimum inhibitory concentration (MIC) was determined following the specified micro-dilution method in the CLSI guidelines (Cockerill *et al.*, 2013). Compound stocks were serially diluted, in triplicate, as follows: azithromycin (512 ng μl^{-1}, 256 ng μl^{-1}, 128 ng μl^{-1}, 64 ng μl^{-1}, 32 ng μl^{-1}, 16 ng μl^{-1}, 8 ng μl^{-1}, 4 ng μl^{-1} and 2 ng μl^{-1}), gentamicin (64 ng μl^{-1}, 32 ng μl^{-1}, 16 ng μl^{-1}, 8 ng μl^{-1}, 5 ng μl^{-1}, 4 ng μl^{-1}, 2.5 ng μl^{-1}, 2 ng μl^{-1}, 1.25 ng μl^{-1} and 0.25 ng μl^{-1}), and curcumin and reserpine (400 ng μl^{-1}, 200 ng μl^{-1}, 100 ng μl^{-1}, 50 ng μl^{-1}, 25 ng μl^{-1}, 12.5 ng μl^{-1}, 6.25 ng μl^{-1} and 3 ng μl^{-1}). Each test included positive and negative growth controls with a DMSO control. The four compounds were dissolved in LB broth + Tween 80 0.5 % (v/v) (Inouye *et al.*, 2001) and 4 % DMSO for curcumin and reserpine. Compound concentrations were tested on bacterial inocula of 5×10^5 CFU ml^{-1} and incubated at 37 °C for 24 hours. The absorbance of each lane was measured with the Multiscan SKY spectrophotometer (Thermo Scientific™, USA).

2.4. Compound mixtures

Compounds were mixed following *mvfR* gene expression assay results with individual compound treatments (see below). Thus, the compound concentration exerting the highest expression inhibition of the *mvfR* gene was considered in each mix. Three compounds were mixed at a time, resulting in three different blends.

2.5. RNA extractions and cDNA synthesis

For RNA extraction, P. aeruginosa was exposed to a $\frac{1}{4}$ of the MICs obtained from inhibition assays with individual compounds and their three mixtures, which included a positive growth control. RNA extraction started when the positive control absorbance was within 0.6 and 0.8 units (i.e., bacterial growth phase values). RNAs were extracted and purified with the RNeasy mini kit (Qiagen, Germany), and DNA degraded with a DNase (Promega, USA), following the manufacturer's instructions.

RNA concentration and purity were determined with a NanoDrop device (Thermo Scientific 2000™, USA) at 260 nm and 260/280 nm, respectively. DNA absence was checked using RNA as a template in a PCR reaction. cDNA was synthesized using an M-MLV reverse transcriptase (Promega, USA) in a 25 µl final volume reaction containing: 1 µg of RNA, 1X buffer RT (100 mM

Tris-HCl (pH 9.0), 500 mM KCl, 1 % Triton® X-100 (Sigma Aldrich, USA), 1.0 mM dNTPs, 20 U of reverse transcriptase, 1.0 µM of random primers according to the manufacturer's instructions. The reaction was incubated for 1 h at 37 °C, followed by 5 min at 95 °C and 10 min at 4 °C.

2.6. Semiquantitative PCR

Housekeeping *rpoD* and *mvfR* gene expressions were assessed via semiquantitative PCR, using the cDNA synthesized. For the *rpoD* gene, forward and reverse primer sequences were: GGGCGAAGAAGGAAATGGTC and CAGGTGGCGTAGGTGGAGAA, respectively. The fragment generated was 178 bp long. For the *mvfR* gene, the forward and reverse primer sequences were GGGCGAAGAAGGAAATGGTC and CAGGTGGCGTAGGTGGAGAA, respectively. The expected amplicon size was 238 bp (Savli *et al.*, 2003; Parai *et al.*, 2018).

The cDNA synthesized (2 µl) was mixed with 1X master mix (Promega®, USA), 1.0 µM primers, and DNase-free water to a final volume of 25 µL. PCR program conditions were 94 °C for 5 min, 35 cycles at 94 °C for 45 s, 55 °C for 45 s, and 72 °C for 1 min, and a final extension at 72 °C for 7 min. PCR products were run on 2 % (P/V) agarose gels and stained with ethidium bromide. Gel images were analyzed by densitometry in the iBrightTM Imaging System, thus obtaining gene expression values from band features for each treatment in triplicate.

2.7. Rhamnolipid and pyocyanin determination

Rhamnolipids were assessed according to the previously described orcinol method (Banerjee *et al.*, 2017). A total of 500 µl of bacterial culture supernatant were collected after 24 hours of growth in LB broth (from each mixture with best results), then mixed with 1.5 ml of diethyl ether and centrifuged at 10 000 rpm. Subsequently, ether-grouped fractions were evaporated at 37 °C, and the remnants dissolved in 100 µl of water and 900 µl of an orcinol solution at 0.18 % (P/V) and 53 % (P/V) sulfuric acid. All samples were heated for 30 minutes at 80 °C in a serological water bath and subsequently brought to room temperature for absorbance reading at 421 nm.

Pyocyanin was quantified following Essar *et al.* (1990). A total of 5 ml of 24-hour growing medium (from each mixture with the best results) were treated with 3 ml chloroform and 1 ml of HCl at 0.2 M and centrifuged at 10 000 rpm. 300 µl of the pink color supernatant were taken for absorbance measurement at 520 nm.

2.8. Statistical analysis

Treatment-dependent *mvfR* gene expression differences were assessed with ANOVA and Tuckey's tests in RStudio. Likewise, differences in rhamnolipid and pyocyanin production among treatments were also evaluated.

3. Results and discussion

The evaluated antibiotics, azithromycin and gentamicin, had minimum inhibitory concentrations of 256 ng μl^{-1} and 5 ng μl^{-1}, respectively (**Figure 1**). Under these two antibiotic concentrations the absorbance of *P. aeruginosa* cultures was below 0.05. None of the tested curcumin and reserpine concentrations elicited P. aeruginosa growth inhibition, as revealed by all absorbance values being above 0.05 (Figure 1C and Figure 1D). Higher concentrations of these natural products were not

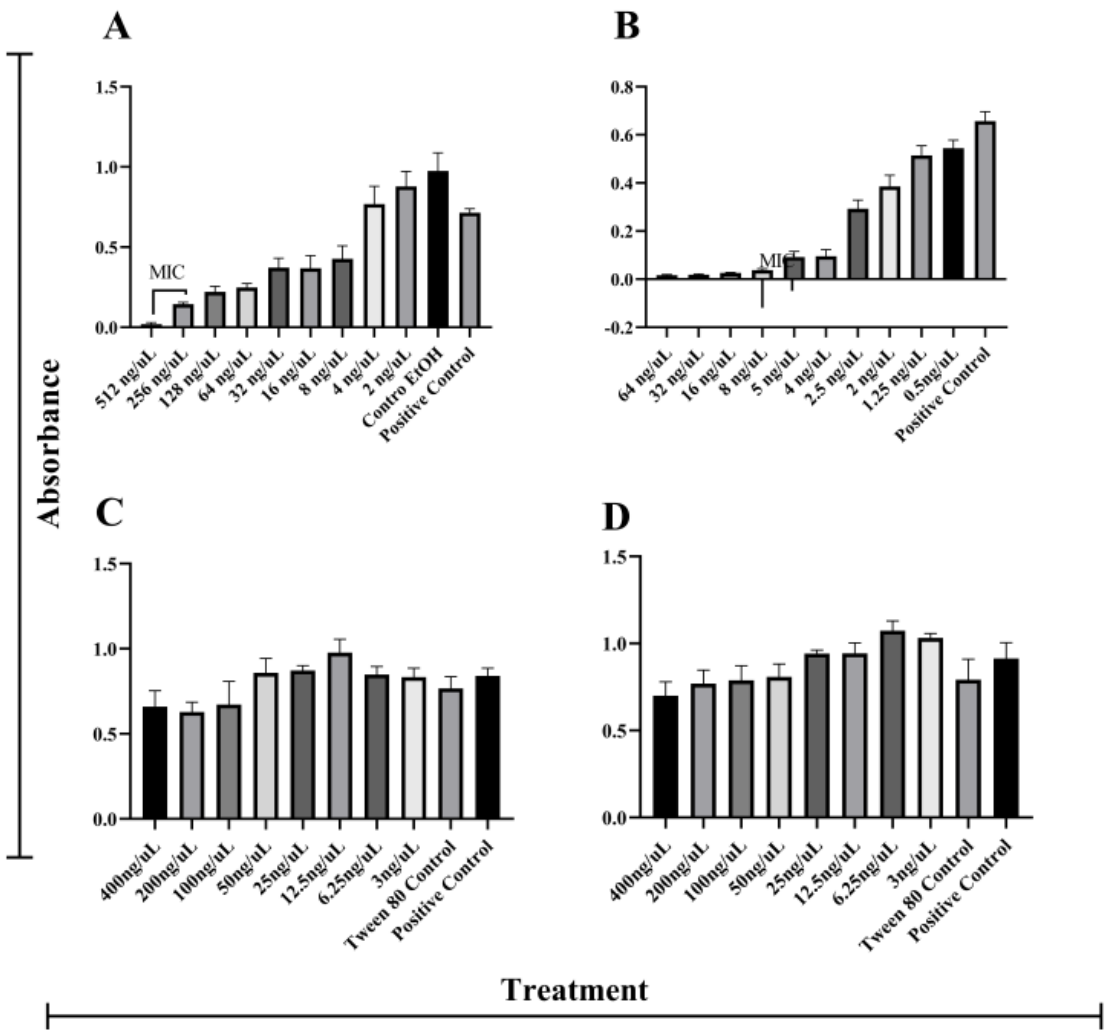

Figure 1. Calculated minimum inhibitory concentrations (MIC) of four tested compounds on *P. aeruginosa* BAA-47 growth. The MIC of each compound was determined as the concentration eliciting a bacterial growth reflected by to an absorbance of 0.05A. (**A**) *P. aeruginosa* growth under variable Azithromycin concentrations. (**B**) *P. aeruginosa* growth under variable Gentamicin concentrations. (**C**) *P. aeruginosa* growth under variable Curcumin concentrations. (**D**) *P. aeruginosa* growth under variable reserpine concentrations. Treatments and control were evaluated under equal conditions. Each assay was performed in triplicate. Bar height represents average absorbance with one standard deviation. The growth bar corresponding to the compound's concertation eliciting a bacterial growth with an absorbance of 0.05A was marked as the treatment's MIC.

evaluated due to their low solubility in culture broth. For this reason, a standard natural compound concentration of 200 ng μl^{-1} was used, as it had sufficient solubility to continue with the RNA extraction process and subsequent evaluation of the expression of the *mvfR* gene.

The expression of the *mvfR* gene, differed significantly among treatments, as shown in **Figure 2**. However, all experienced expression levels below 45 % of that of the control, namely 42 %, 24 %, 26 %, and 25 % for azithromycin, gentamicin, curcumin, and reserpine, respectively. Gentamicin, curcumin and reserpine elicited a stronger *mvfR* gene expression reduction than azithromycin. The housekeeping gene expression did not experience significant variation among treatments and control. This result highlights the fact that treatments only affected the expression of the *mvfR* gene.

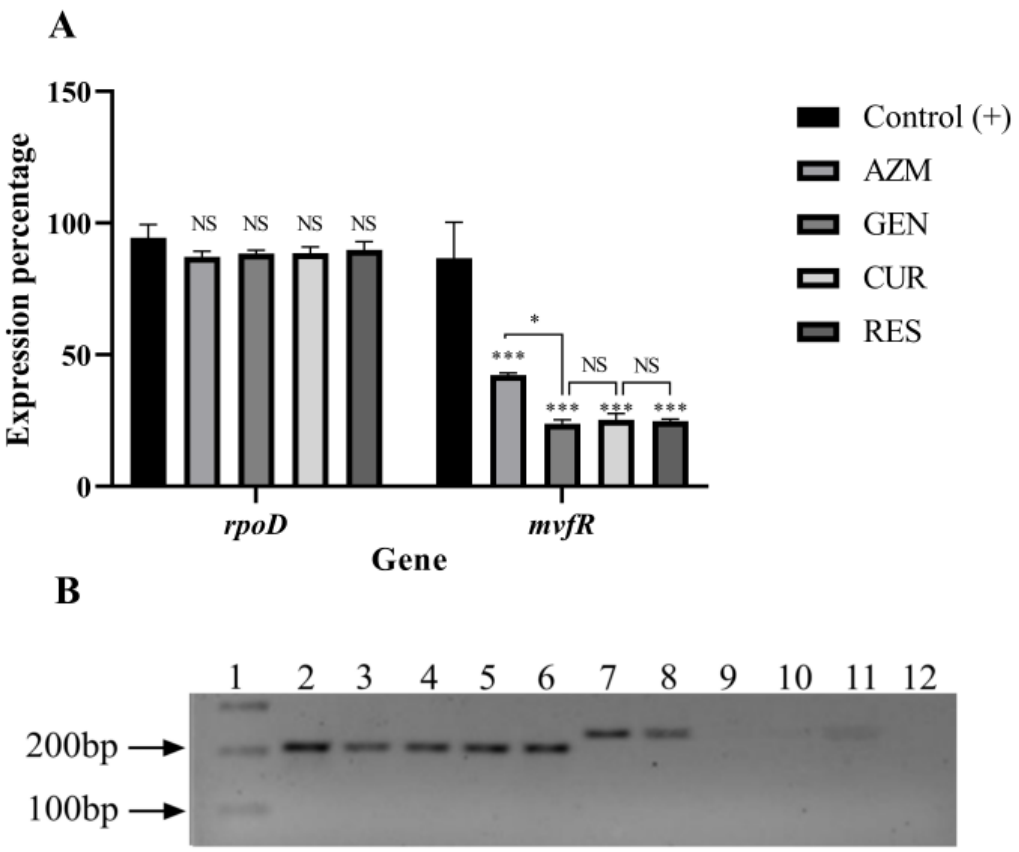

Figure 2. (**A**) Expression of *P. aeruginosa* BAA-47 genes *rpoD* (housekeeping) and *mvfR* genes under azithromycin (AZM), gentamicin (GEN), curcumin (CUR), and reserpine (RES) treatments. The level of expression of each gene under each treatment was established with relation to the positive growth control expression per gene. Bars represent the average (plus standard deviation) of triplicate relative expression levels for each gene and treatment by the semiquantitative analysis in the iBrightTM Imaging System. Significant differences were calculated via ANOVA and Tuckey's test, with three significance levels NS: $P > 0.05$, *: $P \leq 0.05$, and ***: $P \leq 0.001$. NS (Non-significant difference), * (significant difference), *** (very significant difference). (**B**)) Electrophoresis of *rpoD* and *mvfR* PCR products using cDNA as template. Lanes show amplicons under azithromycin, gentamicin, curcumin, and reserpine: 1: M 100 bp, 2: Gene *rpoD* positive control. 3: Gene r*poD* 1/4 MIC azithromycin. 4: Gene *rpoD* 1/4 MIC gentamicin. 5: Gene *rpoD* 200 µg ml^{-1} curcumin. 6: Gene *rpoD* 200 µg ml^{-1} reserpine. 7: *mvfR* gene positive control. 8: *mvfR* 1/4 MIC azithromycin gene. 9: Gene *mvfR* 1/4 MIC gentamicin 10: Gene *mvfR* 200 µg ml^{-1} Curcumin. 11: Gene *mvfR* 200 µg ml^{-1} Reserpine. 12: Reaction target.

The effect of each the three tested the mixtures on *P. aeruginosa* growth is shown in **Figure 3**. The curcumin-reserpine combination affected growth the least, as evidenced by the similarity of its curve with that of the control. The mixtures curcumin-gentamicin and reserpine-gentamicin elicited similar growth effects, revealing lower absorbance values than the positive growth control, evidencing an inhibitory effect on *P. aeruginosa* growth. As seen in Figure 1, neither curcumin nor reserpine exerted such inhibitory effect on their own, so the inhibition elicited by these mixtures is chiefly attributed to gentamicin.

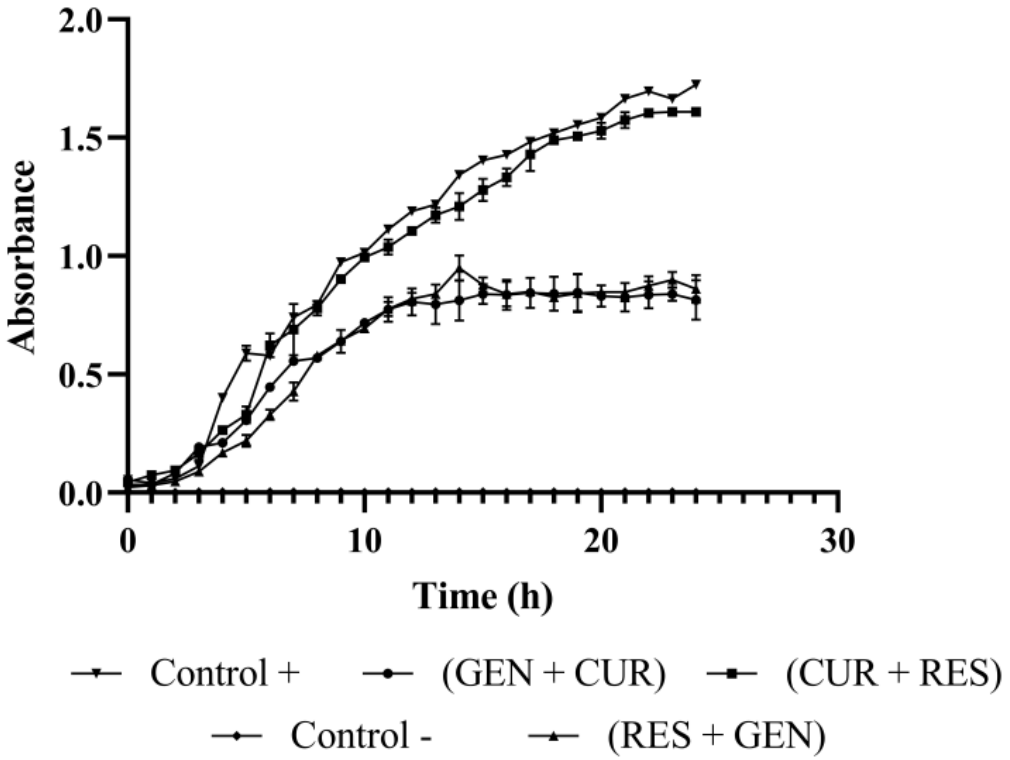

Figure 3. Growth curve of *P. aeruginosa* BAA-47, under mixtures of gentamicin-curcumin (GEN + CUR), curcumin-reserpine (CUR + RES), and reserpine-gentamicin (RES + GEN). Curves show bacterial growth trajectories as revealed by culture absorbances trough time.

The expression percentages of **t**he housekeeping (*rpoD*) and *mvfR* genes under compound mixtures are shown in **Figure 4**A, revealing that the *rpoD* gene expression was not significatively affected by compound mixture, and that *mvfR* gene expression was most affected by the mixture of reserpine and gentamicin, followed the mixture of curcumin and gentamicin. The mixture curcumin with reserpine did not exert a significant inhibition of *mvfR* expression.

As reveled by amplicon band features (Figure 4B) *rpoD* gene expression did not change with any of the mixed compound treatments; whereas, *mvfR* bands (shown in the lanes 8 and 9 in Figure 4B) were absent, implying that the expression of this gene was very low under the curcumin and gentamicin mixture and under the reserpine and gentamicin mixture. Under the mixture between curcumin and reserpine, *mvfR* gene expression was reduced yet perceptible, as revealed by the faint band on lane 7 (Figure 4B).

The three tested compound mixtures modulated pyocyanin and rhamnolipid production. Furthermore, these treatments had a marked effect on pyocyanin than on rhamnolipid levels (**Figure 5**). Gentamicin-reserpine was the best pyocyanin inhibitor, lowering this bacterial virulence factor to 24.5 % of its control (Figure 5A). Curcumin-reserpine resulted in a rhamnolipid level similar to that of its control, implying that this mixture did not inhibit this *P. aeruginosa* virulence factor. However, curcumin-gentamicin significantly lowered rhamnolipid production by 8 %, and reserpine-gentamicin reduced it by 32 % (Figure 5B).

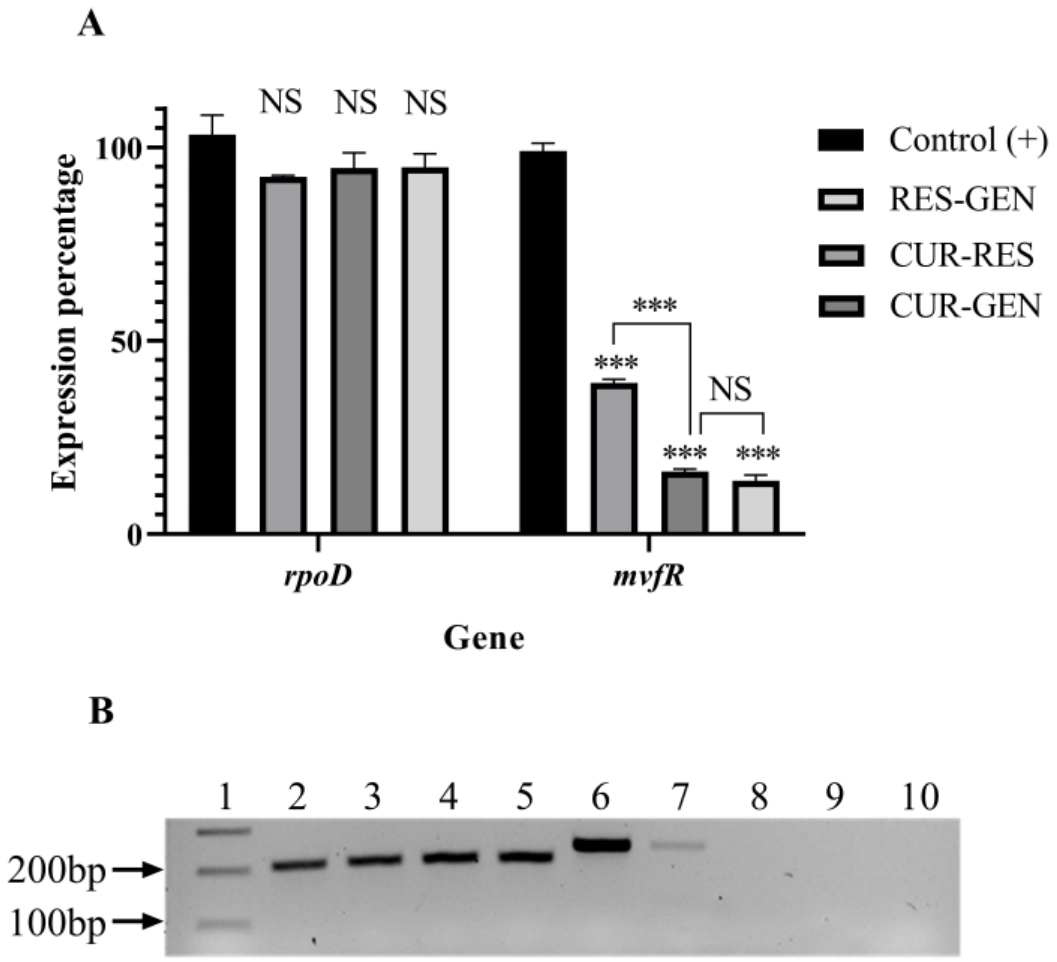

Figure 4. (**A**) Expression of *P. aeruginosa* BAA-47 *rpoD* (housekeeping*)* and *mvfR* genes under reserpine and gentamicin (RES-GEN), curcumin and reserpine (CUR-RES), and curcumin and gentamicin (CUR-GEN) treatments. Gene expression levels are reported relative to the expression observed in the positive growth control per target gene. Each relative expression level (represented by a bar) is the average, plus standard deviation, of technical triplicates subjected to semi-quantitative analysis. Significant differences were calculated with ANOVA and Tuckey tests, with three significance levels: NS: $P > 0.05$, *: $P \leq 0.05$, and ***: $P \leq 0.001$. NS (Non-significant difference), *(significant difference), ***(very significant difference). (**B**) Electrophoresis of *rpoD* and *mvfR* PCR products using cDNA as Template. Lanes show gentamicin-curcumin (GC), gentamicin-reserpine (GR), and curcumin-reserpine (RC) treatments, as follows: 1: M 100 bp, 2: Gene *rpoD* positive control. 3: Gene *rpoD* (CR). 4: *rpoD* (GC) gene. 5: *rpoD* (CR) gene. 6: *mvfR* gene positive control. 7: Gene *mvfR* (CR). 8: *mvfR* (GC) gene. 9: Gene *mvfR* (GR). 10: reaction blank.

The obtained gentamicin and azithromycin minimum inhibitory concentrations (5 ng μl^{-1} and 256 ng μl^{-1}, respectively) coincide with values in previous reports (Bahari *et al.*, 2017). Also, P. aeruginosa standard strain PAO1 and clinical isolates experienced changes in their biofilm formation features in the presence of sub-MIC concentrations of gentamicin compared to controls (no gentamicin). These results revealed that gentamicin at a sub-MIC concentration reduced the expression of genes involved in biofilm formation in P. aeruginosa PAO1 possibly through the effect of the antibiotic on the QS (Davarzani *et al.*, 2021).

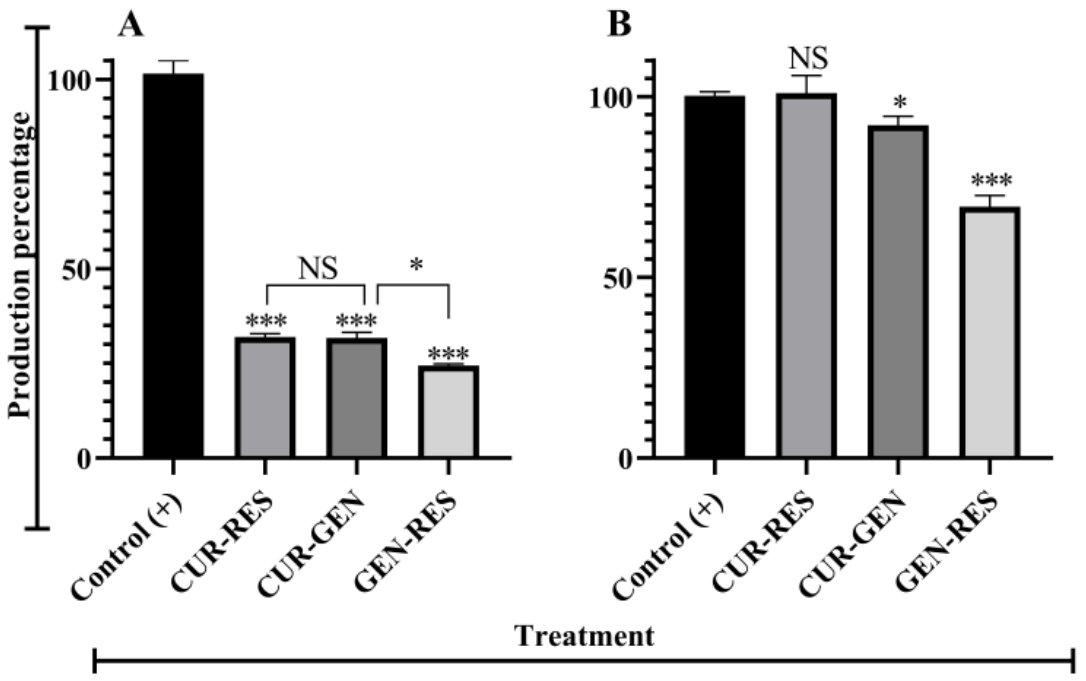

Figure 5. *P. aeruginosa* BAA-47 (**A**) pyocyanin and (**B**) rhamnolipid production levels under three compound-mixtures. The production level of each virulence factor is reported relative to that observed in its positive growth control. The height of each bar is the average, plus standard deviation, of spectrophotometric analysis triplicates. Significant differences were calculated with ANOVA and Tuckey tests, with three significance levels: NS: $P > 0.05$, *: $P \leq 0.05$, and ***: $P \leq 0.001$. NS (Non-significant difference), *(significant difference), *** (very significant difference).

The observed curcumin and reserpine MIC values differ from those previously reported (Ansel *et al.*, 1969; Bahari *et al.*, 2017; Parai *et al.*, 2018), in which minimum inhibitory concentrations for *P. aeruginosa* and Gram-negative bacteria range between 110 ng µl^{-1} and 150 ng µl^{-1} for curcumin and are around 800 ng µl^{-1} for reserpine. In our study it was impossible to reach a minimum inhibitory concentration for these two compounds. This is because in other studies, higher concentrations of organic solvents, such as DMSO, for full dilution were employed to avoid the precipitation of the tested compounds. Our DMSO concentrations were lower (4 % or less) because, as reported by Ansel *et al*., (1969) concentrations above 4 % inhibit bacterial growth by 66 %. This does not allow to determine if the antimicrobial action is caused by the natural compound or by the DMSO. Also, when low DMSO concentrations are used, insoluble compounds in an aqueous environment may appear; for this reason, in this study Tween 80 (0.5 %) was added, which works as surfactant and avoids the precipitation of these compounds at low organic solvent concentrations.

In this study, the changes reported in the *mvfR* gene expression were similar to those revealed by previous reports (Bahari *et al.*, 2017) for treatments with azithromycin and gentamicin and curcumin, exerting significant variation on the expression of lasR/lasI and rhlI/rhlR genes. Also, reserpine has also a well-documented role as inhibitor of pyocyanin and rhamnolipids (Parai *et al.*, 2018).

Curcumin-gentamicin and reserpine-gentamicin mixtures synergistically inhibited *mvfR* gene expression, when compared to the low level of expression achieved by the individual compounds. The synergistic effect of curcumin and gentamicin has been previously reported on the expression

of the lasI/lasR and rhlI/rhlL systems genes (Bahari *et al.*, 2017). An effect in the opposite direction was observed with the curcumin-reserpine mixture, leading to a *mvfR* expression level higher than that with its individual compounds. This concurs with a previous study posing that in natural compound mixtures, their constituents may antagonize each other, nullifying their individual effects on microorganisms (Caesar *et al.*, 2019).

The production levels of *P. aeruginosa* virulence factors pyocyanin and rhamnolipids under gentamicin–reserpine and gentamicin-curcumin mixtures were below those observed under the curcumin-reserpine mixture and the positive growth control. This highlights the last mixture's inadequacy to inhibit these virulence factors and other QS mechanisms, because of the antagonistic effect of the mixture components. The *mvfR* system is related to virulence factors pyocyanin, cyanidin, and lectin, and is also regulated by the rhlR/rhlI system, which regulates rhamnolipid production and biofilm formation. Therefore, the inhibition of PQS system may hinder biofilm formation and the production of virulence factors needed for *P. aeruginosa* infection (Lee and Zhang, 2015).

Changes in the expression of the *mvfR* gene were following reserpine addition. These can be explained by bioinformatics analysis, specifically molecular docking. Molecularly, reserpine competes for the active place of the protein encoded by the *mvfR* gene. When reserpine is docked to that active place, it inhibits the expression of virulence factors corresponding to motility, biofilm formation, rhamnolipids, and pyocyanin, among others. This supports the view that the *mvfR* gene inhibitory effect is related to the interaction between reserpine and the *mvfR* protein's active place and not because of different external factors from treatments (Parai *et al.*, 2018).

Finally, several studies addressing QS mechanisms have demonstrated that they are part of a hierarchical system, where the lasR/lasI system is the modulator of all QS subsystems in *P. aeruginosa*. If lasR/lasI system transcriptional regulators expression is affected, other systems such as the PQS and all virulence factors in *P. aeruginosa* will be affected too (Lee and Zhang, 2015). This is consistent with our results, since gene and virulence factor expression under treatments differed substantially from those of the controls. Furthermore, a synergistic inhibitory effect of curcumin and antibiotics, *e.g.*, gentamicin, on virulence factors of Gram-positive and Gram-negative bacteria has been well documented (Kali *et al.*, 2016). Likewise, other study evidenced synergistic curcumin-ciprofloxacin inhibition on the growth of bacteria associated with urinary tract infections, showing improved performance over the use of antibiotics only (Ameer *et al.*, 2022). Reserpine, in combination with different antibiotics, exerts enhanced inhibitory effects on a wide range Gram-positive and Gram-negative bacterium (Khameneh *et al.*, 2019). This is not the first-time an inhibitory effect of reserpine and curcumin is evidenced, when combined with gentamicin. Considering our results in light of previous findings and their outlook, it would be likely that treatments against *P. aeruginosa* infections will include mixtures of reserpine or curcumin with gentamicin to enhance conventional therapies.

4. Conclusions

The natural compounds reserpine and curcumin and the antibiotic gentamicin had statistically similar inhibitory effects on the QS regulatory gene *mvfR*, outperforming the effect of azithromycin.

Natural compounds and gentamicin mixtures (*i.e.*, reserpine-gentamicin and curcumin-gentamicin) enhanced the inhibition of the *mvfR* gene and *P. aeruginosa* virulence factors, proving their synergistic action. On the other hand, curcumin and reserpine antagonized each other as part of a mix, resulting in insufficient inhibition of *mvfR* gene and its virulence factors expression when compared to the effects of each of these compounds on their own.

The results of this study, together with previous research findings and developments, show that it is possible to have treatments and therapies consisting of mixes between reserpine and curcumin with gentamicin to enhance, or complement, conventional methods to treat infections caused by *P. aeruginosa*.

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6. Conflict of interest

The authors have no conflicts of interest to declare.

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Efectos de compuestos naturales y antibióticos comerciales en la percepción de cuórum de *Pseudomonas aeruginosa*

Resumen: *Pseudomonas aeruginosa* es una bacteria gramnegativa designada por la OMS como microorganismo de prioridad crítica debido a su virulencia, controlada por un sistema de detección de quórum (QS). La QS es regulada por subsistemas específicos: LasI/LasR, RhlI/RhlR y PQS/MvfR. Varios compuestos naturales pueden inhibir estos mecanismos de QS. En este estudio, determinamos el efecto de la curcumina, la reserpina y dos antibióticos comerciales (gentamicina y azitromicina), por separado y combinados, sobre los mecanismos de QS de *P. aeruginosa*: la expresión del gen *mvfR* y la producción de piocianina y ramnolípidos. Las concentraciones inhibitorias mínimas (CIM) de los antibióticos y compuestos naturales se determinaron mediante ensayos de microdilución. La gentamicina, la azitromicina, la curcumina, la reserpina y sus mezclas ejercieron efectos variables sobre la expresión del gen *mvfR*, evaluada mediante ensayos de RT-PCR semicuantitativa. La curcumina, la reserpina y la gentamicina inhibieron la expresión del gen *mvfR* mejor que la azitromicina y las mezclas curcumina-gentamicina y reserpina-gentamicina superaron a la gentamicina sola en cuanto a la inhibición de gen *mvfR* y la disminución en la producción de piocianina y ramnolípidos, revelando el efecto sinérgico de estos compuestos. Las mezclas de curcumina y gentamicina y reserpina y gentamicina pueden convertirse en alternativas para complementar o mejorar los métodos convencionales utilizados actualmente para tratar las infecciones de *P. aeruginosa*.

Palabras Clave: curcumina; Expresión génica; *Pseudomonas aeruginosa*; Percepción de cuórum; Reserpina, Factores de virulencia.

Efeitos de compostos naturais e antibióticos comerciais na percepção do quórum de *Pseudomonas aeruginosa*

Resumo: *Pseudomonas aeruginosa* é uma bactéria gram-negativa designada pela OMS como um microrganismo de prioridade crítica devido à sua virulência, controlada por um sistema de detecção de quórum (QS). A QS é regulada por subsistemas específicos: LasI/LasR, RhlI/RhlR e PQS/MvfR. Vários compostos naturais podem inibir esses mecanismos de QS. Neste estudo, determinamos o efeito da curcumina, reserpina e dois antibióticos comerciais (gentamicina e azitromicina), separadamente e combinados, sobre os mecanismos de QS de *P. aeruginosa*: a expressão do gene *mvfR* e a produção de piocianina e ramnolípideos. As concentrações inibitórias mínimas (MIC) dos antibióticos e os compostos naturais foram determinadas por ensaios de microdiluição. A gentamicina, azitromicina, curcumina, reserpina e suas misturas tiveram efeitos variados na expressão do gene *mvfR*, conforme avaliado por ensaios de RT-PCR semi-quantitativa. A curcumina, reserpina e gentamicina inibiram a expressão do gene *mvfR* melhor que a azitromicina e as misturas curcumina-gentamicina e reserpina-gentamicina superaram a gentamicina sozinha na inibição do gene *mvfR* e diminuição na produção de piocianina e ramnolípideos, revelando o efeito sinérgico desses compostos. Misturas de curcumina e gentamicina e reserpina e gentamicina podem se tornar alternativas para complementar ou aprimorar os métodos convencionais atualmente usados para tratar infecções por *P. aeruginosa*.

Palavras-chave: curcumina; Expressão genética; *Pseudomonas aeruginosa*; percepção do quórum; reserpina, fatores de virulência.

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