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Inhibitory Effect of Five Naturally Occurring Compounds on the Expression of Genes Associated with the QS System and some Virulence Factors in *P. aeruginosa*

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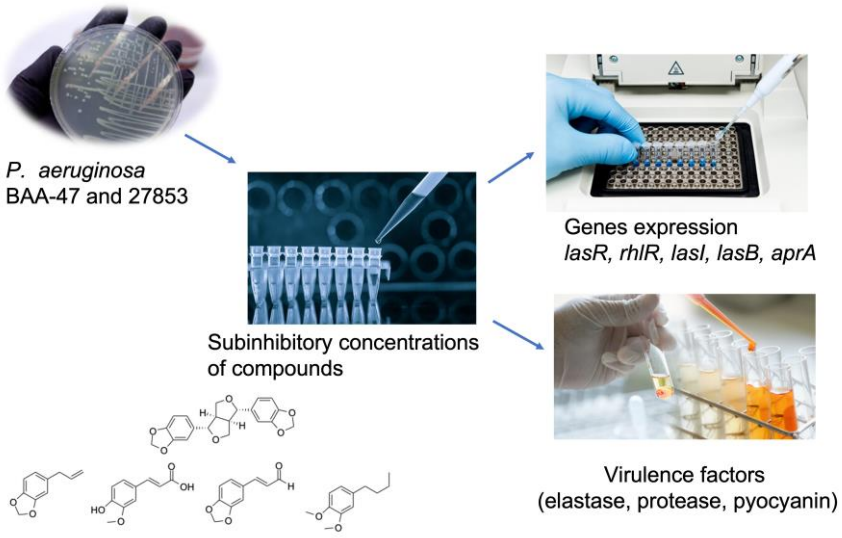
HIGHLIGHTS

- Sesamin and ferulic acid had the highest inhibitory effect on the genes evaluated in *P. aeruginosa*
- These compounds have a possible multitarget effect on the two main systems of QS in *P. aeruginosa.*
- Ferulic acid was the most active compound on two of the three virulence factors evaluated

Abstract: *Pseudomonas aeruginosa* is an opportunistic nosocomial pathogen with a pathogenicity that is an important factor in public health and is the result of the functioning of the Quorum Sensing (QS) system. Currently, compounds of natural origin have shown promising biological effects on certain pathogens in recent years. The aim of the present study was to determine the possible inhibitory effect of five naturally occurring molecules on some genes associated with two pathways of the QS system, as well as on elastase protease and pyocyanin production in two resistant strains of *P. aeruginosa*. For this purpose, RT-qPCR assays were performed to know the expression of some QS-associated genes (*lasR, rhlR, lasI, aprA* and *lasB*) and spectrophotometric assays to evaluate the production of elastase, protease and pyocyanin in the presence or absence of the molecules. It was found that sesamin and ferulic acid were the molecules with the highest inhibitory effect, higher than 40 %, on most of the genes evaluated (*lasR, rhlR, aprA* and *lasB*) in the two *P. aeruginosa* strains BAA-47 and 27853. Likewise, ferulic acid was the most active compound on two of the three virulence factors evaluated even though in general the effect of the molecules was not greater than 50 %. Therefore, these compounds are considered to have a possible multitarget effect by inhibiting the expression of genes associated with the two main systems of *P. aeruginosa.*

Keywords: Quorum sensing; *Pseudomonas aeruginosa*; virulence; natural compounds.

GRAPHICAL ABSTRACT



INTRODUCTION

Pseudomonas aeruginosa was included by the World Health Organization (WHO) in the critical priority group by its high capacity to obtain resistance genes and being a multidrug-resistant pathogen, representing a public health problem, being necessary to develop new antibiotics to treat infections [1–3]. This Gram-negative bacterium is considered an opportunistic nosocomial pathogen causing pulmonary, gastrointestinal, urinary and skin infections, usually affecting hospitalized and immunocompromised patients [4,5].

Its clinical importance relaces to production of multiple virulence factors and the formation of biofilm, result of the functioning of the Quorum Sensing system (QS), which confers specific characteristics for tissue colonization [6,7]. This system is a bacterial communication mechanism based on the synthesis and chemical signaling of autoinducer molecules that regulate gene expression [8,9]. *P. aeruginosa* has three known interconnected QS communication systems: Las, Rhl and PQS, the first two comprises an autoinducer synthase that produces the autoinducer N-3-oxo-dodecanoyl-homoserine lactone (3-oxo-C12-HSL) and N-butanoyl-homoserine lactone (C4-HSL), respectively [10]. However, for the PQS system, there are other types of activators known as 4-hydroxy-2-alkylquinolines (HAQ), within this group are 4-hydroxy-2-heptylquinoline (HHQ) and 2-heptyl-3,4-dihydroxyquinoline (PQS). The synthesis of HHQ requires the presence of the *pqsABCDE* and *phnAB* operons, whereas the synthesis of PQS occurs by the hydroxylation of HHQ by PqsH [10,11]. The inducer 3-oxo-C12-HSL binds to LasR and activates *lasI* and several downstream genes. This complex (LasR-autoinducer) also activates expression of *rhlR* and *rhlI* genes, as well as *pqsR*, *pqsABCDE* and *pqsH* genes [9,12].

Activation of this system allows genes associated with virulence factors to be expressed, being relevant because of their clinical importance; for example, *aprA* (alkaline protease), *lasB* (elastase) and pyocyanin (*pyo*), among others [13,14]. The presence of these multiple virulence factors enhances the pathogenicity of *P. aeruginosa*, favoring the pathogen during the processes of colonization, tissue damage and invasion, and evasion of the host's defense mechanisms [10,15]. A relevant virulence factor is elastase (LasB), the most abundant protein of the secretome of *P. aeruginosa*, which hydrolyzes a high amount of host proteins, causing tissue damage, altering the immune response, and provoking inflammation [16]. For its part, pyocyanin acts on multiple cellular pathways within the host causing ciliary strokes, epithelial dysfunction, neutrophil apoptosis, and consequently, alterations in the lung immune system [17]

Therefore, it is necessary to find alternative therapeutic methods such as the search for QS inhibitors, as a strategy to regulate biofilm formation and decrease the virulence of *P. aeruginosa*, being necessary to know the mechanism of operation of the QS system to determine the route of inhibition of new molecules [18,19]. In the last years, compounds of natural origin have shown promising biological effects on certain pathogens; for example, compounds such as 5-hydroxymethylfurfural, chlorogenic acid and ellagic acid are some molecules that have shown multitarget effects on the genes associated with the QS system *lasI, lasR, rhlI, rhlR* and *pqsR* and on some virulence genes such as *lasB* and *rhlA*, with inhibition percentages between 30 and 70 % for some strains of *P. aeruginosa* [20–22]. It has been widely reported the potential effect of some natural compounds (phenylpropanoids, lignans, terpenes and others) as inhibitors of QS, virulence factors and biofilm on important bacteria in public health [23,24], however, it is necessary to corroborate this

effect on the transcripts or proteins of the QS system in order to report the possible potential for different types of structures previously reported to have other biological effects [10,25]. Therefore, the aim of the present study was to determine the possible inhibitory effect of five molecules of natural origin on some genes associated with two pathways of the QS system, as well as on of elastase, protease and pyocyanin production in two resistant strains of *P. aeruginosa*.

MATERIAL AND METHODS

Compound specifications

The compounds evaluated (5,5'-(1S,3aR,4S,6aR)-tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diylbis(1,3-benzodioxol) (sesamin) 1, *trans* -3- (4´-hydroxy-3´-methoxyphenyl)prop-2-enoic acid (ferulic acid) 2, trans-3-(4',5'-methylendioxyphenyl)prop-2-enal 3, 1,2-dimethoxy-4-propylbenzene 4, and 5-(2´-propenyl)-1,3-benzodioxole) (safrole) 5. Of the five compounds evaluated, three (sesamin, ferulic acid and safrole) are commercial with purity greater than 94%; and the remaining compounds are synthesis products of methyleugenol (1,2-dimethoxy-4-propylbenzene) and safrole (*trans*-3-(4',5'-methylendioxyphenyl) prop-2-enal), Figure 1. The selection of compounds was derived from a previous literature review of molecules with possible effect on the Quorum Sensing system and previous results of the effect of the compounds on biofilm formation [26]. This allowed to stablish the concentrations of the compounds, Table 1.

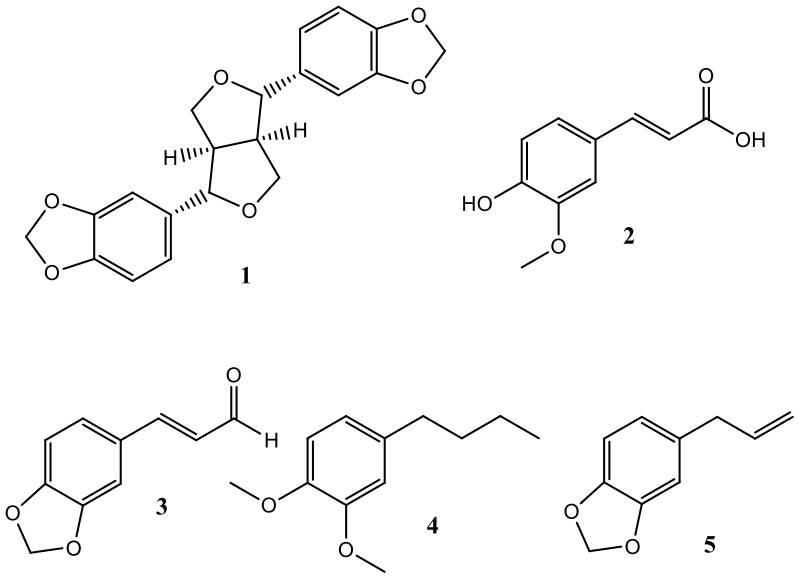


Figure 1. Compounds evaluated on the gene expression and virulence factors on *P. aeruginosa*. Sesamin **1**, ferulic acid **2**, trans-3-(4',5'-methylendioxyphenyl) prop-2-enal **3**, 1,2-dimethoxy-4-propylbenzene **4**, and safrole **5**.

Table 1. Concentration of the five molecules to be evaluated on the two strains of *P. aeruginosa*

Compound number	IUPAC name (Common name)	Concentration (µg/mL)	
		ATCC BAA-47	ATCC 27853
1	5,5'-(1S,3aR,4S,6aR)-tetrahydro-1H,3H-furo[3,4-c]furan-1,4-diylbis(1,3-benzodioxole) (sesamin)	25	25
2	*trans* -3- (4´-hydroxy-3´-methoxyphenyl) prop-2-enoic acid (ferulic acid)	1,5	50
3	*trans*-3-(4',5'-methylendioxyphenyl) prop-2-enal	13	50
4	1,2-dimethoxy-4-propylbenzene	25	25
5	5-(2´-propenil)-1,3-benzodioxole) (safrole)	3	100

Bacterial strains and culture

The reference strains used were *P. aeruginosa* ATCC BAA-47 and ATCC 27853. The culture was performed in Luria Bertani (LB) broth and incubated on an orbital shaker (Jeio Tech IST-3075) at 37 °C, 180 rpm for 24 h. The method used to preserve the morphological, physiological, and genetic characteristics of the strains was freezing at -70 °C in 20% glycerol and LB.

Determination of the effect of molecules with QS inhibitory potential on the expression of some genes of the Las and Rhl systems in two strains of P. aeruginosa.

Primer selection and verification

The transcriptional receptor genes of the two main QS systems (*lasR* and *rhlR*) and three cascade genes related to virulence factors (*lasI, aprA* and *lasB*), as well as the housekeeping gene (*rpoD*) were selected. The primers corresponding to these genes were selected from the literature Table 2, verifying their amplification for subsequent use in expression studies. For conventional PCR, 1X GoTaq® Green Master Mix (Promega USA), 0.5 µM primers (Table 2), 2 µL of template and DNase-free water were used for a final volume of 25 µL. The amplification conditions used in the thermal cycler were: 94 °C for five minutes (denaturation), then 35 cycles of 94 °C for 45 seconds, 55 °C for 45 seconds and 72 °C for one minute with a final extension at 72°C for one minute. The amplification products were run on a 2% agarose gel (P/V). Additionally, real-time PCR amplification efficiency evaluation was performed for each gene to avoid overestimation of gene expression in the assays [27–29].

Table 2. Genes evaluated with the sequence corresponding to the primers used and the respective expected products in bp

Gene	Sequence	Expected product size (pb)	Reference
rpoD	F- GGGCGAAGAAGGAAATGGTC R-CAGGTGGCGTAGGTGGAGAA	178	[30]
lasR	F-ACGCTCAAGTGGAAAATTGG R- GTAGATGGACGGTTCCCAGA	247	[31]
rhlR	F- AGGAATGACGGAGGCTTTTT R- CCCGTAGTTCTGCATCTGGT	231	[31]
lasI	F- CTACAGCCTGCAGAACGACA R- ATCTGGGTCTTGGCATTGAG	168	[31]
lasB	F- GGAATGAACGAAGCGTTCTC R- GGTCCAGTAGTAGCGGTTGG	300	[32]
aprA	F- CCCTGTCCTATTCGTTCCTG R- GCGTCGACGAAGTGGATATT	175	[33]

RNA extraction and *RT-qPCR*

To determine the effect of the five compounds on gene expression, an overnight culture of *P. aeruginosa* was diluted 1:100 in LB medium and incubated with the molecules or DMSO at 1% (control) until the growth phase equivalent to an optical density at 600 nm between 0.6 and 0.8, that is approximately 1×10^9 CFU/mL [34]. Each assay was performed in triplicate. For the extraction of total RNA, the RNeasy® Mini kit Qiagen (USA) was used together with RNA protect (Qiagen USA) for the stabilization and protection of the extracted RNA, additionally to eliminate the contaminating DNA in the RNA samples, RNase-free DNase I "RQ1 RNase-Free DNase (Promega USA)" was used. Purity was verified by conventional PCR on RNA and the concentration of each sample was measured using the NanoDrop (Thermo Fisher USA). For reverse transcription, between 0.8 to 1.0 µg of RNA was used using the same amount in the control (RNA extracted from bacteria without molecule but with DMSO) and treatments and cDNA was synthesized, using the M-MLV enzyme (Promega) and random primers (0.5µg) (Promega USA) in a final volume of 25µL. Finally, real-time qPCR was performed using the CFX96™ Real-Time PCR kit (Bio-Rad USA) and the Luna® Universal qPCR (USA) Master Mix kit using 2 µL of cDNA with a primer concentration of 0.25 µM. The final amplification conditions were as follows: a single cycle at 95 °C for 1 minute (polymerase activation) and 38 amplification cycles of 95 °C for 30 seconds (denaturation) and 62 °C for 30 seconds (hybridization and extension) and a final cycle corresponding to the 60-95°C melting curve. The amplification profiles and melting curves were analyzed and the threshold cycle (Ct) values for the *lasR, rhlR* and affected genes (*lasI, lasB* and *aprA*) (Table 2) were normalized to the Ct of the *rpoD* housekeeping gene [33,35,36]. Differential gene expression was plotted using the mathematical model that calculates gene expression changes as a relative change in gene expression (as seen by SYBR® Green fluorescence) between treatments and the housekeeping gene (*rpoD*) Ct ($2^{-\Delta\Delta Ct}$) [37].

Evaluation of molecules on the production of QS-associated virulence factors in two strains of *P. aeruginosa*

Additionally, the selected molecules were evaluated for their effect on elastase, protease and pyocyanin production at the same concentrations used in the gene expression studies. From an overnight culture, independently, each of the treatments was centrifuged at 10,000 rpm for 15 minutes to separate the supernatant. Each of these assays was performed in triplicate and the results were expressed as percent formation according to the following equation [38]. The positive control was bacterium with 1% DMSO and each treatment or 1 % DMSO without bacterium were considered as a blank.

$$\% \text{ de formation or production} = (\text{OD treatment}/ \text{OD bacterium control})*100$$

Protease quantification assay

75 µL of the overnight culture supernatant was taken and mixed with 0.3 % azocasein in buffer solution (100 mM Tris pH 8.0), this mixture was incubated at 37 °C for 30 minutes. The reaction was stopped using 10 % trichloroacetic acid, then centrifuged at 10,000 rpm for 10 minutes. Finally, the supernatant was taken and the protease activity was read spectrophotometrically at 400 nm [39].

Elastase quantification Assay

50µL of overnight culture supernatant was mixed with 450 µL of buffer (100 mM Tris pH 7.5) containing elastin congo red (ECR) (20 mg/mL) and incubated at 37 °C for 3 hours with shaking. After incubation, the reaction was stopped with 500 µL of phosphate buffer saline (PBS 0.7 M pH 6.0), centrifuged at 10,000 rpm for 10 minutes to remove insoluble ECR. Finally, elastase activity was measured at 495 nm [39].

Pyocyanin quantification assay

750 µL of overnight culture supernatant and 350 µL of chloroform were placed, the tubes were shaken strongly with the help of a vortex until the release of a blue color was observed, then they were left to stand for 4-5 minutes where the two phases were separated. The organic phase containing this blue pigment was taken and acidified with 300 µL of 0.2 M HCl, again shaken vigorously until a slight color change to pink was observed. Spectrophotometric measurements were taken at 520 nm [40].

Statistical analysis

All values obtained (percent formation) were presented as mean ± standard deviation (SD). A one-way ANOVA analysis was performed considering the assumptions of independence, homogeneity and randomness on the absorbance measurements obtained in the assays (positive control, treatments, and negative control). Subsequently, for these same, a Shapiro-Wilk normality test and a Duncan's multiple comparison test between means were performed. A p-value < 0.05 was considered to indicate significant differences [38].

RESULTS

The compounds sesamin and ferulic acid showed an inhibitory effect on *lasR* and *rhlR* genes in the two strains tested and *aprA* and *lasB* cascade genes in strain BAA-47. The compounds 1,2-dimethoxy-4-propylbenzene, *trans*-3-(4',5'-methylendioxyphenyl) prop-2-enal inhibited the *lasR* and *lasB* gene in the two *P. aeruginosa* strains and *rhlR* and *lasI* only in strain 27853. Additionally, it was possible to determine that safrole was the compound with the least inhibitory capacity on gene expression in the two *P. aeruginosa* strains evaluated (Figure 2,3).

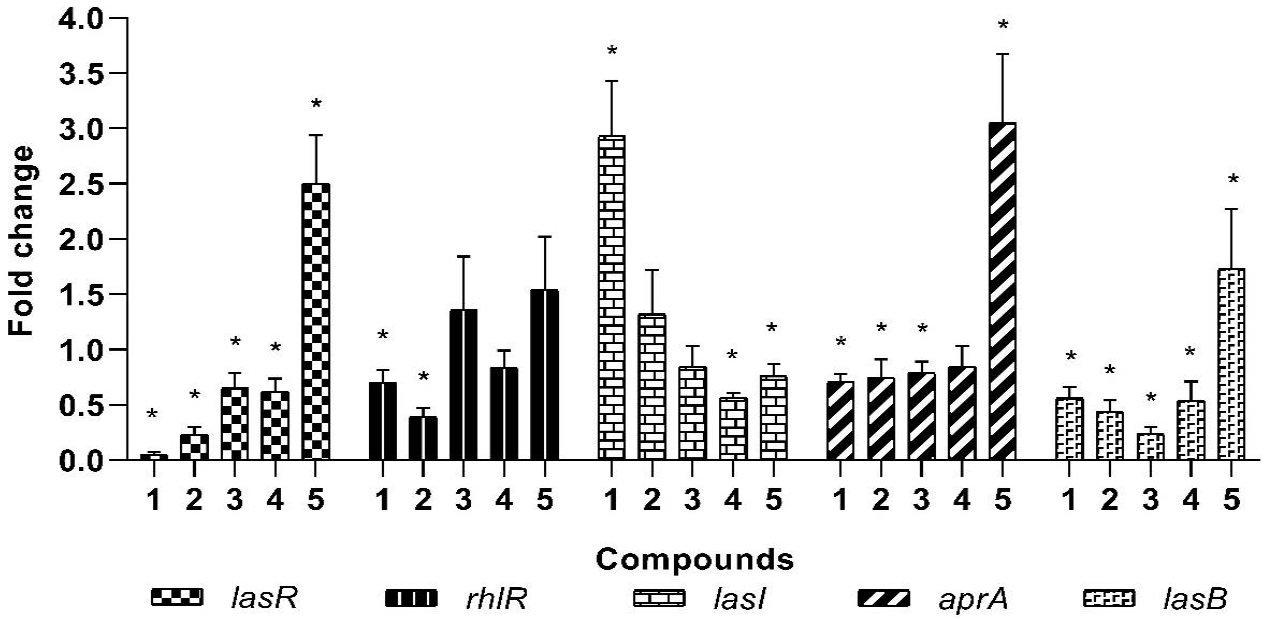


Figure 2. Effect of the five compounds evaluated on the expression of *lasR, rlhR, lasI, aprA* and *lasB* genes on *P. aeruginosa* BAA-47 by RT-qPCR, the *rpoD* gene was used as a calibrator and the expression was normalized by the $2^{-\Delta\Delta CT}$ method. *Treatments that are significantly different from the control (one-way ANOVA followed by the Duncan test; P<0.05). Sesamin **1**, ferulic acid **2**, *trans*-3-(4',5'-methylendioxyphenyl) prop-2-enal **3**, 1,2-dimethoxy-4-propylbenzene **4**, and safrole **5**.

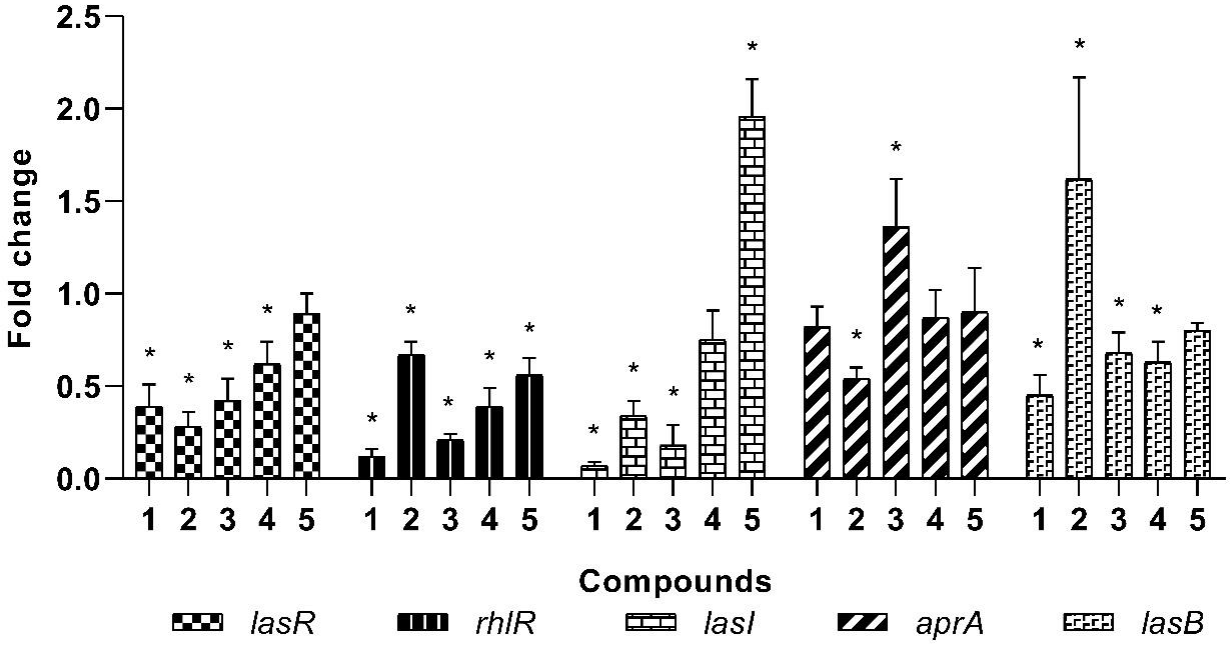


Figure 3. Effect of the five compounds evaluated on the expression of *lasR, rlhR, lasI, aprA* and *lasB* genes on *P. aeruginosa* 27853 by RT-qPCR, the *rpoD* gene was used as a calibrator and the expression was normalized by the $2^{-\Delta\Delta CT}$ method. *Treatments that are significantly different from the control (one-way ANOVA followed by the Duncan test; P<0.05). Sesamin **1**, ferulic acid **2**, *trans*-3-(4',5'-methylendioxyphenyl) prop-2-enal **3**, 1,2-dimethoxy-4-propylbenzene **4**, and safrole **5**.

Figure 4 and 5 shows the results obtained for the five compounds evaluated on protease, elastase and pyocyanin production for the two strains of *P. aeruginosa*. It was found that none of the compounds significantly inhibited protease in strain ATCC BAA-47, while sesamin was the only compound that showed

significant effect in strain ATCC 27853. On the other hand, the compounds safrole, ferulic acid, trans-3-(4',5'-methylendioxyphenyl)prop-2-enal and 1,2-dimethoxy-4-propylbenzene showed significant effects on elastase production in strain ATCC BAA-47, while 1,2-dimethoxy-4-propylbenzene was the only molecule able to significantly affect elastase production in strain ATCC 27853. Additionally, it was determined that sesamin favored the production of elastases in the two strains evaluated. With respect to the production of pyocyanin, the molecules evaluated did not show to be very active against this virulence factor; however, it was determined that sesamin and ferulic acid were the compounds that presented the greatest effect on the production of pyocyanin (approximately 20.5%).

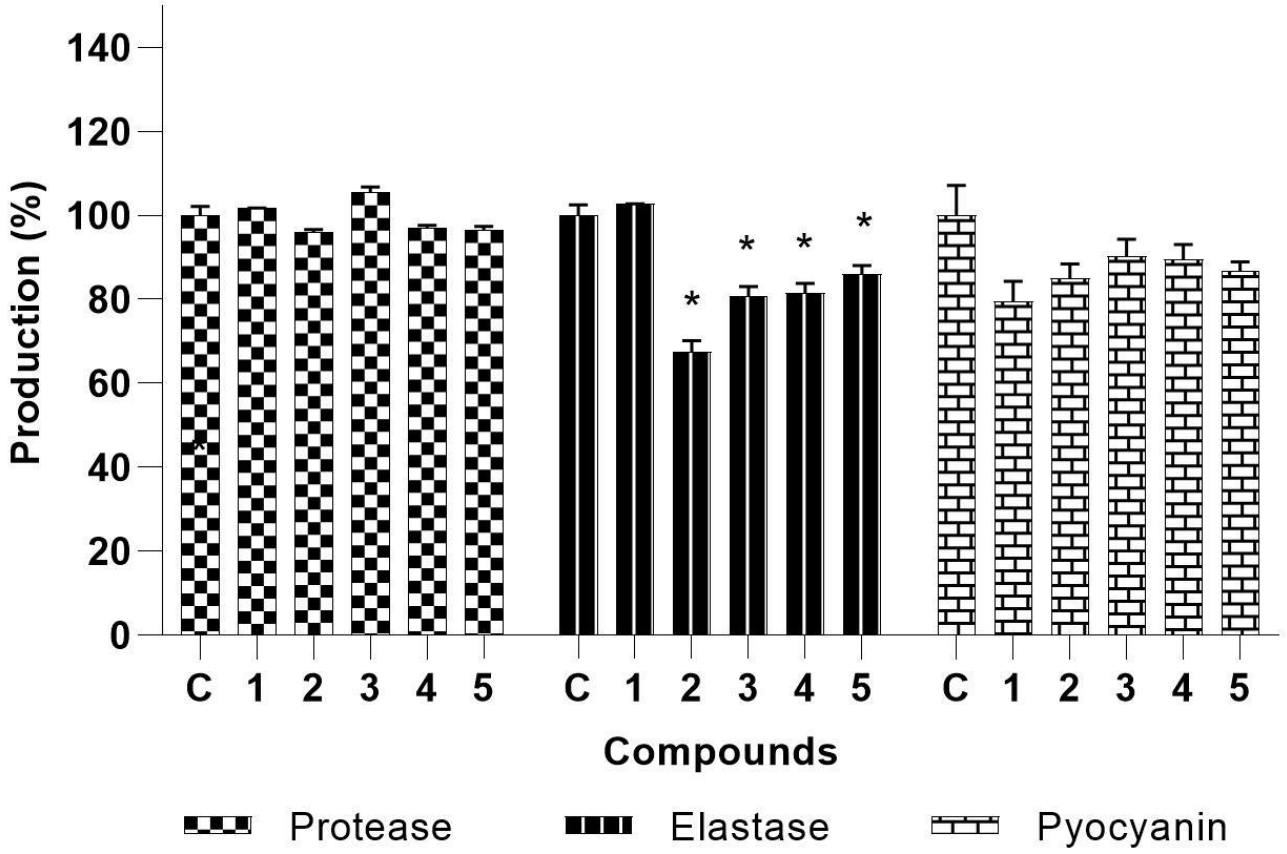


Figure 4. Effect of the five compounds evaluated on the production of *P. aeruginosa* (ATCC BAA-47) virulence factors Analysis of variance (ANOVA) significant effect* ($p < 0,05$). C(+) (bacterium with 1% DMSO), sesamin **1**, ferulic acid **2**, *trans*-3-(4',5'-methylendioxyphenyl) prop-2-enal **3**, 1,2-dimethoxy-4-propylbenzene **4**, and safrole **5**.

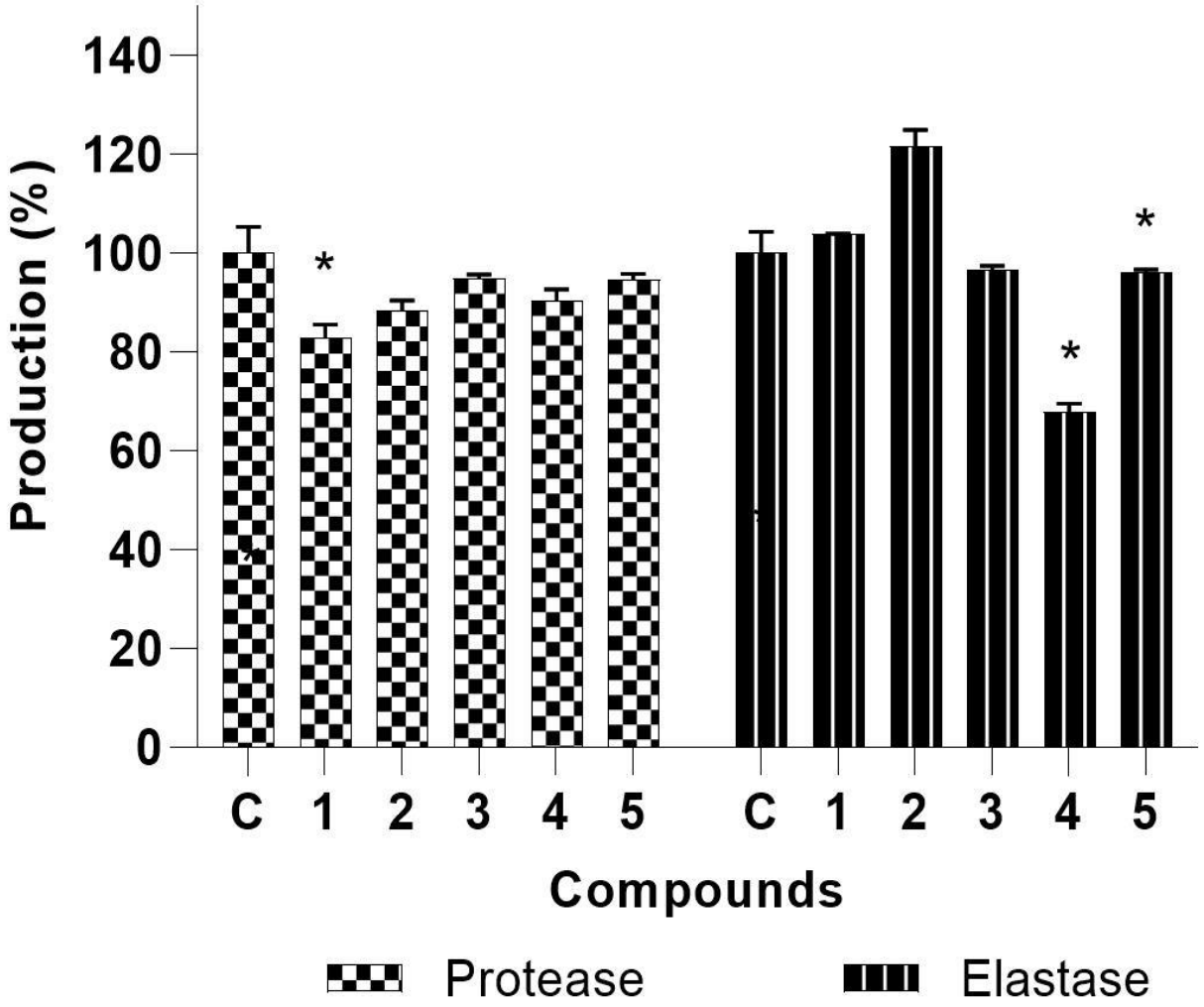


Figure 5. Effect of the five compounds evaluated on the production of *P. aeruginosa* (ATCC 27853) virulence factors. Analysis of variance (ANOVA) significant effect* ($p < 0,05$). C(+) (bacterium with 1% DMSO), sesamin **1**, ferulic acid **2**, *trans*-3-(4',5'-methylendioxyphenyl)prop-2-enal **3**, 1,2-dimethoxy-4-propylbenzene **4**, and safrole **5**.

DISCUSSION

P. aeruginosa is currently one of the most studied pathogens because it occurs with the highest incidence in chronic infections. Its virulence behavior is regulated by QS, hence, the continuous search for new molecules and their effect on the QS system [20]. This study highlights the importance of knowing and verifying the effect of compounds of natural origin on the QS system of *P. aeruginosa*, responsible for the activation of multiple virulence factors in this pathogen.

The sesamin and ferulic acid compounds showed an inhibitory effect on the expression of the genes of the Las and Rhl systems of the two strains evaluated. From the five evaluated compounds there is only one report about sesamin in molecular experiments [33], and for the four remaining there had not been reports of its effect on genes studied. For sesamin, inhibition greater than 80% in the *lasR, rhlR and lasI* genes in strain PAO1 has been reported [33], being similar to that found in strain ATCC 27853 in the present study. Molecular docking studies have reported that their inhibition on genes may be associated with the coupling with the LasR and LasI protein through hydrophobic interactions, and in turn with the expression of the cascade virulence factor associated with the LasR and LasI system [33].

Ferulic acid was the most active compound in the evaluation of gene expression, mainly, it had the capacity to inhibit the expression of the *lasR* gene between 71-77 % and the *rhlR* gene between 49-57 % in both strains. The gene inhibition of the two systems are relate to previous reports that show that *lasR* gene favors the autoinduction of this same system and in turn activates the second pathway with the *rhlR* gene at

both the transcriptional level and the posttranslational level [14,41]. This simultaneous inhibitory effect on the two systems was also observed for the compounds trans-3-(4',5'-methylendioxyphenyl) prop-2-enal, 1,2-dimethoxy-4-propylbenzene, and sesamin against the ATCC 27853 strain. This effect is consistent with reports previous for quercetin [42] , derivatives of benzimidazole and baicalin among others [43]. Ferulic acid has no previous reports on genes evaluated but its potential on QS system has been proposed, for example, there is a report of inhibition between 35-40% on the LasR protein in a bioluminescence assay with mutant strains [44] and inhibition of the *tetK, msrA* and *norA* genes on *Staphylococcus aureus* strains (RN4220, IS-58 and SA1199B) [45,46]. Also, there is a recent report with *in silico* studies, where it is suggested that ferulic acid is a potential inhibitor of the transcriptional regulator RhlR in *P. aeruginosa* [47].

Trans-3-(4',5'-methylendioxyphenyl)prop-2-enal showed an opposite effect in the ATCC strain BAA-47 where there was inhibition of the *lasR* gene but not on the expression of the *rhlR* gene. These results can be explained by the relation with the third system, PQS [14] , and presence of alternative regulatory systems that modulate the expression of some genes, for example, the transcriptional regulators Vfr and GacA that activate the *lasR* and *rhlR* genes, directly intervening in the cascade expression of the QS system [48,49] .

Additionally, the results of ferulic acid and *trans*-3-(4',5'-methylendioxyphenyl) prop-2-enal, especially in strain 27853, showed a decrease in the expression of *lasI* close to 60% and 80%, respectively. It is likely that the action of this compound is on the LasR protein and in turn on the *lasR* gene, which is not being transcribed at the same level as the control; this would explain why the formation of the transcriptional receptor to which the autoinducer signal should bind is not favored. A study showed this effect using 2-Hydroxyanisole, which had a high probability of union to LasR *in silico*, and in transcriptomics analysis were observed that the compound down regulated *lasR* and cascade genes like *lasI* and *lasB*, between others [50].

Considering the systematic regulation of QS in *P. aeruginosa*, it was possible to corroborate the effect that molecules on the systems (Las and Rhl) have on the expression of genes associated with virulence factors. For example, it was corroborated that the compounds sesamin, in BAA-47 strain, ferulic acid and trans-3-(4',5'-methylendioxyphenyl) prop-2-enal, in both strains, inhibited the *lasR* gene and the *aprA* cascade gene with inhibition percentages between 20-45 %. A report showed a similar effect where Terpinen-4-ol decrease the expression of QS genes (*lasI, lasR, rhlI, rhlR*) and virulence factors genes (*lasB, aprA, toxA*, and *plcH*), and in turn it was confirmed by its effect directly on virulence factors [51]. However, sesamin inhibited the *lasR* gene in strain 27853, but not the *aprA* gene. This effect was previously reported by other authors where sesamin inhibited 78.5 % *lasR* gene expression, however, *aprA* gene expression was not affected, probably because of the alternative regulatory systems present in *P. aeruginosa* that not only activate but can also switch off the expression of other genes associated with the system [33].

Another cascade gene, associated with the system, is *lasB* whose expression is controlled by the Las and Rhl system, this cascade effect was found with the compounds sesamin, *trans*-3-(4',5'-methylendioxyphenyl) prop-2-enal and 1,2-dimethoxy-4-propylbenzene for the two bacteria evaluated, ferulic acid with strain BAA-47 and safrole in strain 27853 (Figure 2 and 3). This effect has also been reported by Li and coworkers, who reported a significant inhibition of 80% by the diallyl disulfide compound on the *rhlR* gene and a total inhibition (98%) on the *lasB* [52]. However, the compound *trans*-3-(4',5'-methylendioxyphenyl) prop-2-enal, in BAA-47 strain, showed no inhibitory effect on the *rhlR* gene but did show an inhibitory effect on the expression of *lasB* gene, which may be related unique to its effect on the Las system. Similarly, it has been possible to corroborate inhibitory effects by other compounds that show a similar trend to that reported in the present study but that use other methodologies such as gene silencing, RNAseq or measurement of Miller units by measuring β-galactosidase activity, such as that reported for molecules derived from 3-nitrophenol where reporters and modified strains of *Escherichia coli* are used [53,54].

The molecules evaluated presented greater inhibition in elastase production, especially for safrole, ferulic acid, trans-3-(4',5'-methylendioxyphenyl) prop-2-enal and 1,2-dimethoxy-4-propylbenzene showed significant effects in strain ATCC BAA-47, while sesamin and ferulic acid presented the greatest effect on the production of pyocyanin (approximately 20.5%). It is important to highlight that the values for strain ATCC 27853 were not included, since it presented low values of absorbance to be quantified.

The compounds ferulic acid, *trans*-3-(4',5'-methylendioxyphenyl)prop-2-enal, 1,2-dimethoxy-4-propylbenzene and safrole do not present previous reports of their inhibitory activity on the three virulence factors (protease, elastase and pyocyanin) on *P. aeruginosa*, being this their first report. However, in a previous study it was found that ferulic acid produces an inhibition of 32.4% (250 g/mL) on *lasA* protease in the staphylolytic assay and 50.29% in pyocyanin production [55].

In the present study Sesamin showed inhibition of virulence factors only in elastase, while a report show inhibition in various virulence factors as elastase, protease and pyocyanin in the PAO1 strain at 75 μg/mL [33], these differences are explained for concentration (25 μg/mL). In the same study it was determine that

sesamin showed inhibition in the expression of genes associated with the Las and Rhl systems and in the expression of some virulence factors such as protease, similar to a previous report [33].

In this research was possible to corroborate the relationship between the expression of some genes with some virulence factors, for example, ferulic acid and sesamin in strain ATCC BAA-47 showed inhibitory effects of the Rhl system in the case of the quantification of *lasB* gene and elastase (Figure 2 and Figure 4), evidencing and corroborating the activation of cascade genes and virulence factors; similar to previous reports [41].

Finally, it is possible to highlight that some phenolic compounds have previous reports of inhibition for some virulence factors and genes associated with QS in *P. aeruginosa*. For example, it has been reported that trans-cinnamaldehyde inhibits swarming by about 50% and was shown in silico to have a binding affinity for the LasI protein and coumarin significantly inhibits protease production, pyocyanin, biofilm formation and the expression of genes such as *lasI* and *rhlI* [56,57]. Additionally, the effect of this type of compounds has also been reported on virulence factors and their associated genes in other microorganisms. These compounds include quercetin, which has reported inhibitory activity on biofilm formation, some virulence factors (motility) and expression of QS genes (*avrA, hilA, rpoS and luxS*) in *Salmonella enterica* [58]; as well as 2,3-dimethylHQ and 2,5-ditertbutylHQ showed significant inhibition of the expression of *Vibrio parahaemolyticus* QS-associated genes such as *luxS* and *luxI*, and some virulence factors such as toxins, pili, phosphatases, outer membrane channels, among others (*toxR, aphA, mshA, tolC*) [59] .

Considering the above results, the structures of the compounds with the best inhibitory effect, ferulic acid, sesamin and 1,2-dimethoxy-4-propylbenzene, associated with the QS genes, could be considered for future studies on the synthesis of alternative molecules that enhance the effect found, as future drug alternatives to treat *Pseudomonas* mediated infections possibly in conjunction with antibiotics.

CONCLUSION

The results of this study allow to conclude, sesamin and ferulic acid were the compounds that showed the greatest inhibitory effect on *lasR and rhlR* genes, as well as their effect on *lasI, aprA* and *lasB* cascade genes in the two strains evaluated. Similarly, ferulic acid and 1,2-dimethoxy-4-propylbenzene showed an inhibitory effect on elastase and protease production in the two *P. aeruginosa* strains. The inhibition of the Las and Rhl system genes by these compounds determines a multitarget effect and allows postulating sesamin and ferulic acid as alternative molecules for the development of new drugs against infections caused by *Pseudomonas.*

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