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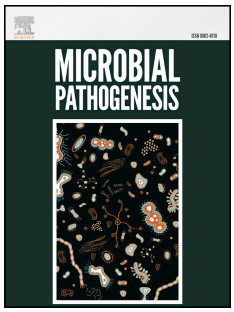


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# Bioavailability-enhanced Resveramax™ modulates quorum sensing and inhibits biofilm formation in *Pseudomonas aeruginosa* PAO1

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**Abstract**

Quorum sensing (QS), a cell-to-cell communication mechanism present in many bacterial species plays a key role in regulating the virulence factor and biofilm formation in many pathogens, which causes severe public health risk. Therefore, interfering with QS mechanism offers an attractive strategy to combat bacterial infections. In the present study, anti-QS activity of a novel resveratrol formulation, Resveramax™, was detected using *Chromobacterium violaceum* biosensor bioassay and the effect of Resveramax on QS-regulated phenotypes in *Pseudomonas aeruginosa* PAO1 was assessed by standard protocols. Molecular docking analysis of resveratrol, the major active constituent of Resveramax, with LasR receptor protein was performed to evidence the QS-inhibitory potential of Resveramax. Results showed that Resveramax specifically inhibited the QS-mediated violacein pigment production in *C. violaceum*; pyocyanin production, proteolytic activity, swarming motility and biofilm formation in *P. aeruginosa* PAO1 in a concentration-dependent manner. Biofilms treated with Resveramax showed increased susceptibility to antibiotics when compared with the antibiotic treatment alone. Molecular docking analysis proved that resveratrol binds more rigidly with LasR receptor protein with docking score of -8.55 KJ/mol. These findings suggest that Resveramax could be used as novel QS-based antibacterial/anti-biofilm agent for the management of bacterial infections.

**Key words:** Quorum sensing inhibition; Resveratrol; Biofilm; Antibiotics; *Pseudomonas aeruginosa.*

## 1. Introduction

The quorum sensing (QS) mechanism enables bacteria to detect their population density through the production, release, and perception of small diffusible signal molecules called autoinducers and to coordinate gene expression accordingly [1,2]. A wide array of functions in bacteria ranging from bacterial cell motility to complex behaviors such as biofilm formation and production of virulence factors are regulated by QS in many pathogenic bacteria [3,4]. It is estimated that 80% of all bacterial infections are biofilm related. The growth of bacteria in biofilms can enhance their resistance to antimicrobial agents and cause a variety of infections including chronic lung, wound and ear infections [5]. Hence, interfering with QS mechanism is being viewed as an alternative strategy to inhibit bacterial infections and QS inhibitors paved the way for the development of novel compounds as chemotherapeutic agents [6]. Unlike antibiotics, anti-QS drugs target biochemical processes which are not essential for bacterial survival, but are important for their pathogenesis and thereby minimize the emergence of drug resistant pathogens [7]. *N*-acyl homoserine lactone (AHL) and oligopeptides are the most common QS signal molecules present in Gram-negative and Gram-positive bacteria respectively and have been targeted to discover anti-pathogenic properties of natural products [8].

In the quest for QS inhibitors, studies have demonstrated that many synthetic and natural compounds exhibit anti-QS activity [9]. However, limitations with the synthetic compounds led to a search of natural plant-derived QS inhibitors for their potential use in various applications. Plant-derived compounds have been used to treat microbial infections for centuries and are supposed to be safe for human consumption [10]. Compounds such as ajoene from garlic, catechin from *Combretum albiflorum* and iberin from horseradish have shown various extents of anti-biofilm and QS-inhibitory properties against several pathogens specifically inhibited QS in

reporter strains [11,12]. Screening of plant-derived compounds with improved strategy may facilitate the discovery of compounds that attenuate bacterial pathogenesis/biofilms. The use of modern computational techniques like molecular docking studies, based on the three-dimensional structure of the target protein, aid in the identification of novel lead molecules by predicting the optimal conformation and key interactions of promising small molecules to its receptor with high binding affinity and minimal toxicity [13]. Recently, long alkyl chain containing phenolic structural motifs (zingerone, (6)-shogaol, (6)-gingerol) present in *Zingiber officinale* has been identified as inhibitors of QS against *Chromobacterium violaceum* and *Pseudomonas aeruginosa* PAO1 [14].

Resveratrol, a polyphenolic compound present in many plant extract has been reported to possess numerous biological functions including antibacterial activity [15], anti-inflammatory, anticancer [16] and cardioprotective activities [17]. The highest concentration of resveratrol has been reported in *Polygonum cuspidatum*, commonly known as knotweed [18]. Resveratrol has been used as a dietary nutrient and approved as Generally Recognized as Safe (GRAS) by Food and Drug Administration [19]. When taken orally, resveratrol is well absorbed by humans but its bioavailability is relatively low because it is rapidly metabolized and eliminated [20]. To increase the bioavailability of resveratrol, a novel formulation of resveratrol i.e., Resveramax™ (50% resveratrol, 1% sunflower lecithin and 1% grape seed oil) has been designed. In the present study, we investigated the anti-biofilm and anti-QS activity of Resveramax against *Chromobacterium violaceum* 12472 and *Pseudomonas aeruginosa* PAO1.

## 2. Materials and methods

### *2.1. Bacterial strains and culture conditions*

Bacterial strains, *Chromobacterium violaceum* 12472 and *Pseudomonas aeruginosa* PAO1 were procured from Microbial Type Culture Collection and Gene Bank, Chandigarh, India. All the bacterial strains were cultured aerobically in Luria-Bertani (LB) medium (1% tryptone, 0.5% yeast extract, 0.5% NaCl) with 120 rpm agitation in a shaking incubator at 37 ºC for 24 h, except *C. violaceum* which was incubated at 30 ºC. Tryptic soy broth (TSB) was used for biofilm studies. LB swarm agar was supplemented with 0.6% (w/v) agar and D-glucose was added (5 g/L filter sterilized and added separately). All media were prepared using deionized water.

*2.2. Minimum inhibitory concentration of Resveramax*

The minimum inhibitory concentration (MIC) of Resveramax was determined for each organism as per the guidelines of clinical and laboratory standards institute, USA. Briefly, 1% of overnight grown cultures (0.4 OD@600nm) were added to microtitre plates containing appropriate growth medium supplemented with Resveramax to attain the final concentration ranging from 1-1000 µg/mL. Plates were incubated overnight and recorded for MIC as the lowest concentration that showed complete inhibition of visible growth. All further experiments in the present study were performed only at sub-MIC concentrations of Resveramax.

*2.3. Biosensor Bioassay*

QS inhibitory potential of Resveramax was detected by biosensor bioassay using *C. violaceum* 12472 [21]. Overnight grown culture was added to LB semisolid agar medium, poured onto sterile petriplate and allowed to solidify. Sterile discs (6 mm, Himedia) loaded with different concentrations of Resveramax was placed on the surface of agar medium and incubated at 30 ºC for 24 h. Plates were observed for an obscure, colorless zone of violacein pigment inhibition around the discs. Dimethyl sulfoxide (DMSO) was used as negative control.

*2.4. Quantification of violacein inhibition*

Inhibition of violacein production in *C. violaceum* 12472 by Resveramax was quantitatively analyzed as described previously with modification [22]. Briefly, 10 mL LB broth containing different concentrations of Resveramax (25, 50, 100 and 200 µg/mL) was inoculated with 100 µL of *C. violaceum* 12472 (0.4 OD@600nm) and incubated for 24 h under shaking (120 rpm). After incubation, an aliquot of 1 mL culture was centrifuged (8000 rpm for 10 min) and supernatant was discarded. 1 mL of water saturated n-butanol was added to the cell pellet to extract the violacein. The mixture was vortexed vigorously for 2 min, allowed to stand for 30 min and centrifuged (8000 rpm for 10 min) to remove the cells. Violacein extracted in butanol was quantified spectrophotometrically at $OD_{585}$. Simultaneously, to test the effect of Resveramax on bacterial growth, culture grown in presence of Resveramax was serially diluted and plated on LB agar medium. After incubation for 24 h, bacterial number was enumerated using a colony counter.

*2.5. Inhibition of pyocyanin production*

*P. aeruginosa* PAO1 (0.4 OD@600nm) was grown in LB medium supplemented with different concentrations of Resveramax (25, 50, 100 and 200 µg/mL) and incubated for 24 h. Pyocyanin from the cell free supernatant (5 mL) was extracted with chloroform (1:3 v/v) and subsequently, the chloroform layer was acidified with 0.2 M HCl, giving it a pink to deep red color. Pyocyanin-containing acid layer was separated and quantified by recording $OD_{520}$ spectrophotometrically [23].

*2.6. Inhibition of swarming motility*

Overnight grown cultures of *P. aeruginosa* PAO1 (0.4 OD@600nm) were placed (5 µL) on swarm agar surface supplemented with different concentrations of Resveramax (10, 25 and 50

µg/mL) and allowed to dry for 30 min at room temperature. Swarm plates were incubated at 37 ºC for 24 h and swarm diameters were measured [24].

*2.7. Antibiofilm activity of Resveramax*

The quantification of biofilm formation using regular 96 well microtitre plates was performed as described previously with minor modifications [11]. *P. aeruginosa* PAO1 was grown overnight in LB medium at 37 ºC with agitation. After growth, the culture was diluted with TSB medium ($OD_{600}$ of ~0.02) and 50 µL of the diluted culture was added to fresh TSB medium in 96 well polystyrene microplates supplemented with different concentration of Resveramax (25, 50, 100 and 200 µg/mL) and incubated statically for 20 h at 37 ºC. After incubation, planktonic cells were discarded, and the biofilms were washed three times with phosphate buffered saline (PBS) and fixed with methanol. After 15 min, the methanol was discarded, and the tubes were dried at room temperature. Crystal violet (0.1% in water) was then added to stain the biofilms and incubated for 15 min. Crystal violet was then discarded, and stained biofilms were washed three times with water. Acetic acid was added to the stained biofilms to solubilize the crystal violet, and the absorbance of the solution was read at $OD_{590}$ spectrophotometrically.

In addition to crystal violet based quantification of biofilm biomass, cell viability within biofilms exposed to Resveramax was determined using the formazan dye-based MTT assay [25]. Briefly, biofilms were grown in 96 well microplates for 20 h as described above, in the presence and absence of Resveramax. After incubation planktonic cells were removed and the remaining biofilm was gently rinsed three times with PBS. After rinsing, 100 µL of PBS and 10 µL of the MTT labeling reagent were added and the suspension was incubated for 4 h at 37 ºC, followed by addition of 100 µL of solubilization solution. The absorbance measurements were taken using a plate reader at 570 nm.

*2.8. Synergistic effect of Resveramax with antibiotics*

The synergistic effect of Resveramax with antibiotics, ciprofloxacin and tobramycin, on the *P. aeruginosa* biofilms was assessed on the 20 h-old biofilm. For this, the bacteria (0.4 OD@600nm) were cultured in TSB media and incubated for 20 h. After incubation, planktonic cells were discarded and the biofilm was rinsed gently with sterile PBS. Fresh media containing antibiotic alone or Resveramax alone or a combination of an antibiotic and Resveramax was added to the biofilm containing plates and incubated at 37 ºC for 20 h. Control set was maintained by adding only fresh media. After 20 h, planktonic cells were discarded and the biofilms were rinsed with PBS. Fresh media was added and incubated at 37 ºC for 20 h. The viable cells in the culture were counted by standard plate count method and compared with control set.

Simultaneously, in another set, bacterial culture (0.4 OD@600nm) was treated with Resveramax and allowed to grow statically for 20 h. After incubation, it was treated with same concentration of antibiotics as above. After 20 h, planktonic cells were discarded and the biofilms were rinsed with PBS. Fresh media was added and incubated at 37 ºC for 20 h. The viable cells in the culture were counted by standard plate count method and indicated as viable cells in the biofilm.

*2.9. Fractional inhibitory concentration*

Synergistic effect resulted from combining Resveramax with antibiotics was assessed by determining fractional inhibitory concentration (FIC) index. FIC was determined by the formula: FIC index = FIC A + FIC B = [A]/MIC A + [B]/MIC B, where [A] is the concentration of drug A, MIC A is it's MIC and FIC A is the FIC of drug A for the organism, while [B], MIC B and FIC B are defined in the same fashion for drug B. The FIC index thus obtained was interpreted

as follows: <0.5, synergy; 0.5 to 0.75, partial synergy; 0.76 to 1.0, additive effect; >1.0 to 4.0, indifference; and >4.0, antagonism [26]. Finally, the varying rates of synergy between the agents were determined.

*2.10. Docking*

Molecular docking analysis was performed to identify the conformational changes in the protein structure. AutoDock tool was utilized to generate grids, calculate dock score and evaluate the conformers of inhibitor bound in the active site of enzyme LasR as target for QS inhibitory activity. Automated docking is a graphical user interface. AutoDock 4.2 was employed to get docking and binding scores; which is implemented by Lamarckian genetic algorithm method. The structure of ligand molecule Resveratrol was analyzed using ACD/Chemsketch. The PRODRG server was used to minimize energy of drug compound and 3D coordinates were prepared. The protein structure file (PDB ID: 2UV0) was taken from PDB and edited by removing the hetero atoms using Python molecule viewer. The grid map was centered at particular residues of the protein and was generated with AutoGrid. As per genetic algorithm all the torsions were allowed to rotate during docking. The Lamarckian genetic algorithm and the pseudo-Solis and Wets methods were applied for minimization, using default parameters [27].

*2.11. Statistical analysis*

All the experiments were performed in triplicates and the data are presented as mean ± standard deviation. Analysis of variance was conducted and differences between variables were tested for significance by one-way ANOVA with Tukey's test using the SPSS program. Differences at $p < 0.05$ were considered statistically significant.

**3. Results**

*3.1. Minimum inhibitory concentration of Resveramax*

Growth profile of *C. violaceum* 12472 and *P. aeruginosa* PAO1 in the presence of different concentration of Resveramax was studied. MIC was determined as the lowest concentration that showed complete inhibition of visible growth. MIC of Resveramax was found to be 500 µg/mL for *C. violaceum* 12472 and 1000 µg/mL for *P. aeruginosa* PAO1. Sub-MIC of Resveramax, not showing any significant growth inhibition (10-100 µg/mL for *C. violaceum* 12472 and 10-200 µg/mL for *P. aeruginosa* PAO1) was selected and used for further experiments.

*3.2. Resveramax inhibits quorum sensing in C. violaceum*

QS inhibitory activity of Resveramax was indicated by loss of purple pigmentation in *C. violaceum* biosensor bioassay (Fig. 1). At 10 µg/disc, Resveramax exhibited 14 mm diameter zone of violacein inhibition. As the concentration increases, there was an increase in the size of halo zones around the discs. However, no activity was evident at the concentration of 1 µg/disc of Resveramax. The QS inhibitory activity of Resveramax and resveratrol was not significantly different in biosensor bioassay. Both resveratrol and Resveramax exhibited 20 mm diameter zone of violacein inhibition at the concentration of 100 µg/disc (Fig. S1). Though the anti-QS activity was similar for both Resveramax and resveratrol, Resveramax was selected because of its enhanced bioavailability. Vehicle controls (1% sunflower lecithin and 1% grape seed oil) did not show anti-QS activity (Fig. S1).

*3.3. Quantification of violacein inhibition*

Resveramax exhibited significant reduction in QS-mediated violacein pigment production in *C. violaceum* 12472 in a concentration-dependent manner. At 75 µg/mL, Resveramax showed inhibition of violacein by more than 90% without affecting bacterial growth (Fig. 2). Complete

inhibition of violacein was observed at 100 µg/mL, however, there was a slight reduction in bacterial growth. Resveramax was able to inhibit violacein production by more than 30% at the concentration of 25 µg/mL when compare to control and more than 70% inhibition of violacein was observed at the concentration of 50 µg/mL.

*3.4. Resveramax inhibits virulence factor production and biofilm formation in P. aeruginosa PAO1*

Inhibition of pyocyanin production, swarming motility and biofilm formation that are associated with QS in *P. aeruginosa* PAO1 were investigated at different concentrations of Resveramax. Results indicated that inhibitory activity of Resveramax against pyocyanin production is concentration-dependent. Resveramax, at the concentration of 200 µg/mL, inhibited pyocyanin production by more than 95% compare to control without significantly inhibiting the growth (Fig. 3).

*P. aeruginosa* PAO1 grown in presence of Resveramax showed significant reduction in QS-mediated swarming motility (Fig. 4). At 50 µg/mL of Resveramax, *P. aeruginosa* PAO1 exhibited swarming zone of 16 mm as compared to control of 72 mm. Inhibition of swarming motility was observed even at the concentration of 10 µg/mL. *P. aeruginosa* PAO1 growth was not affected at the tested concentrations, but features indicative of swarming were completely absent. Interestingly, Resveramax inhibited swarming motility in *P. aeruginosa* PAO1 but promoted its green pigment production, a QS-controlled phenotype.

Biofilm formation in *P. aeruginosa* PAO1 is partially controlled by QS phenomenon in which cells are organized in a matrix of mucoid polysaccharides. Concentration-dependent decrease in biofilm formation in *P. aeruginosa* PAO1 was observed upon treatment with

Resveramax. Biofilm formation was reduced by 90%, 75% and 50% respectively at 200, 100 and 50 µg/mL of Resveramax (Fig. 5).

*3.5. Resveramax increases susceptibility of P. aeruginosa PAO1 biofilms to antibiotics*

Synergistic activity of Resveramax with antibiotics, ciprofloxacin and tobramycin, on *P. aeruginosa* PAO1 biofilms was assessed. *P. aeruginosa* PAO1 treated with Resveramax and antibiotics showed significant reduction in biofilm formation compare to antibiotic and Resveramax treatment alone. Pre-treatment of *P. aeruginosa* biofilms with Resveramax (100 µg/mL) for 20 h and then with antibiotics, ciprofloxacin (2 µg/mL) and tobramycin (4 µg/mL), exhibited significant biofilm inhibitory activity compare to combination of Resveramax and antibiotic post-treatment (Table 1). FIC index was calculated for each dose of treatment and different combination of antibiotics. Results showed that combination of Resveramax (100 µg/mL) with ciprofloxacin (1 µg/mL) and tobramycin (2 µg/mL) was synergistic (FIC < 0.5). Both ciprofloxacin and tobramycin exhibited FIC index of 0.225.

*3.6. Molecular docking analysis*

Molecular docking analysis was carried out using AutoDock program to study the binding potential of resveratrol in the active site of LasR protein. The three-dimensional structure of LasR protein was retrieved from PDB database. LasR receptor protein contains 9 α helices, four β strands along with three β hairpin turns. Second β hairpin turn is important as it forms the active site cavity of this protein. The active site residues are interacting with resveratrol to change its conformation for functioning activity. The results indicated that resveratrol exhibited docking score of -8.55 kJ/mol in the active pocket of LasR protein. Mainly the interaction was Van der Waal force of attraction along with the formation of three hydrogen bonds primarily

with Asp73, Thr75, Leu125 residues of LasR protein (Table 2). The pose of resveratrol in LasR receptor protein was shown in Fig. 6.

**4. Discussion**

Many bacterial species use QS system as their intercellular signaling mechanism, which is found to be implicated in various functions including bacterial pathogenicity, biofilm formation and food spoilage [28]. Hence, inhibition of QS has emerged as a novel approach to target pathogenic bacteria. The dietary polyphenol resveratrol has been shown to have many beneficial effects in humans and animal models [29]. Though resveratrol exhibited high oral absorption, its bioavailability is very low because of rapid and extensive metabolism, resulting in only trace amounts of unchanged resveratrol in the systemic circulation [20]. A novel formulation of resveratrol i.e., Resveramax which contains 50% resveratrol has been designed to increase its bioavailability. In the present study, Resveramax was evaluated for its potential anti-QS and anti-biofilm activities in *C. violaceum* and *P. aeruginosa* PAO1. In *C. violaceum*, the LuxR homolog, CviR regulates the production of a purple pigment violacein (30). *P. aeruginosa* PAO1 utilizes two interrelated QS systems, LasI/R and RhlI/R, to control several genes involved in biofilm formation and virulence factor production such as LasB elastase, LasA protease, pyocyanin, rhamnolipids and exotoxins (31).

Anti-QS activity of Resveramax was initially screened against *C. violaceum* biosensor strain. Resveramax exhibited a concentration-dependent reduction in violacein production indicated by zone of pigmentation loss. In a quantitative study, Resveramax showed more than 90% reduction in violacein at the concentration of 75 µg/mL and it was confirmed that inhibition of violacein production was not because of antibacterial activity. By comparison, catechin inhibited more than 80% violacein inhibition at the concentration of 1000 µg/mL [11]. Similarly, other authors

have demonstrated that polyphenol compounds such as gallic acid, epigallocatechin gallate and curcumin can interfere with bacterial QS system [32,33,34].

*P. aeruginosa* PAO1 is one of the opportunistic human pathogen having well established QS mechanism to control the expression of a number of genes involved in the production of virulence factors and biofilm formation [35]. Swarming motility plays an important role in *P. aeruginosa* infections mainly by enhancing the rapid surface migration and colonization of host cells and also involved in formation of biofilms. Resveramax significantly inhibited swarming migration without inhibiting their growth, proving that the swarming inhibition by Resveramax was not due to an antibacterial effect. Inhibition of swarming motility could aid in reducing the biofilm formation in *P. aeruginosa* by interfering with the ability to reach the substratum. Another QS-controlled virulence factor involved in pathogenicity of *P. aeruginosa* such as pyocyanin pigment production was also significantly reduced by Resveramax. Pyocyanin plays a role in pulmonary tissue damage observed with chronic lung infections and also contributes in a variety of ways to the pathophysiological changes observed in airways infected by *P. aeruginosa* [35]. Resveramax might have down regulated the genes involved in these virulence factor productions either by degrading the AHL molecules or inhibiting the binding of AHL-LasR protein complex to the promoter region. It has been reported that resveratrol inhibits swarming motility and virulence factor expression in *Proteus mirabilis*, a pathogen infecting urinary tract and thereby inhibit the ability of *P. mirabilis* to invade human urothelial cells [36].

The ability of clinical isolates of *P. aeruginosa* to form biofilms is associated with the capacity of these organisms to survive within hospital environments, on implanted medical devices, and in the wounds of patients [37]. Bacteria within biofilms reach a much higher cell density than their planktonic counterpart. As QS inhibitors are known to limit biofilm formation

to a therapeutically relevant degree, it could be used to prevent colonization or as adjunct therapy to enhance the therapeutic efficacy of conventional antibiotics [38,39]. The bacterial cell surface properties like charge and hydrophobicity also play a crucial role in bacterium-host cell interactions. Changes in cell surface charge and reducing hydrophobicity will expose the bacterial biofilm to antibiotics and sequentially will ease the eradication of the biofilm [40]. Resveratrol is a potent inhibitor of biofilm formation in *Vibrio cholerae* and *P. aeruginosa* [41,42]. Transcriptome analysis showed that resveratrol suppressed the expression of QS-induced genes *lasI, lasR, rhlI* and *rhlR* in *P. aeruginosa* PAO1 [43].

In the combined treatment, when the *P. aeruginosa* cells are incubated statically along with antibiotic and Resveramax, biofilm formation was significantly reduced when compare to antibiotic alone. In another experiment, initial treatment of *P. aeruginosa* PAO1 cells with Resveramax and subsequent treatment with antibiotics significantly reduced the biofilm viability almost by double the activity of antibiotic and Resveramax post-treatment. Similar to the present study, the compounds of garlic inhibit *P. aeruginosa* biofilms and also make the biofilm susceptible to antibiotics such as tobramycin [44,45]. Additionally, ajoene, as well as the furanone C-30, was shown to synergize with the antibiotic tobramycin in eradicating *P. aeruginosa* biofilms and aid in the clearance of *P. aeruginosa* from lungs in a mouse model of pulmonary infection [12,46]. It has been reported that furanone compounds penetrate microcolonies and block cell signalling in most biofilm cells as revealed by green-fluorescent protein-based analysis, however it did not affect initial attachment to the abiotic substratum but it does affect the architecture of the biofilm and enhances the process of bacterial detachment, leading to a loss of bacterial biomass from the substratum [46]. QS and signal transduction in biofilms might be much more complicated because of a range of physical, chemical and

nutritional factors that may influence signal production, stability, distribution and efficiency to interact with their cognate receptors in a biofilm.

Structure based virtual screening provides valuable information to design targeted drugs. In the present study, we have performed the molecular interaction analysis of resveratrol, the major active constituent in Resveramax, with LasR receptor protein of *P. aeruginosa*. Molecular docking analysis of LasR receptor protein showed that resveratrol binds rigidly to the receptor with docking score of -8.55 KJ/mol. The strong interaction between the compounds may be due to the particular specific groups, which mediates conformational changes in the receptor protein. In a recent study, trans-resveratrol from grape vine exhibited docking score of -9.5 Kcal/mol and -7.8 Kcal/mol in the active site pocket of LasR and CviR protein respectively [14]. El-Mowafy et al. evidenced the quorum quenching potential of aspirin through molecular docking analysis [47]. The present study evidenced *in vitro* and *in silico* that Resveramax efficiently inhibited QS-regulated phenotypes in *C. violaceum* 12472 and *P. aeruginosa* PAO1, which offers a potential candidate for the development of novel therapeutic agents against multi drug-resistant bacteria.

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Table 1: Synergistic activity of Resveramax with antibiotics, ciprofloxacin and tobramycin against *P. aeruginosa* PAO1 biofilms.

| Treatments | Log CFU/mL | |
|---|---|---|
| | Treatment 1* | Treatment 2** |
| Untreated control | 9.47 | 9.12 |
| Cip(2) | 5.79 | 5.82 |
| Tob(4) | 5.35 | 5.26 |
| R | 5.62 | 5.15 |
| Cip(1)+ R | 3.47 | 2.32 |
| Cip(2) + R | 2.18 | 1.24 |
| Tob(2) + R | 3.29 | 1.78 |
| Tob(4) + R | 1.96 | 1.17 |

Cip, Ciprofloxacin; Tob, Tobramycin; R, Resveramax (100 µg/mL).

*20 h-old biofilms were treated with a combination of antibiotic and Resveramax.

** Biofilms were treated with Resveramax for 20 h and then treated with antibiotics.

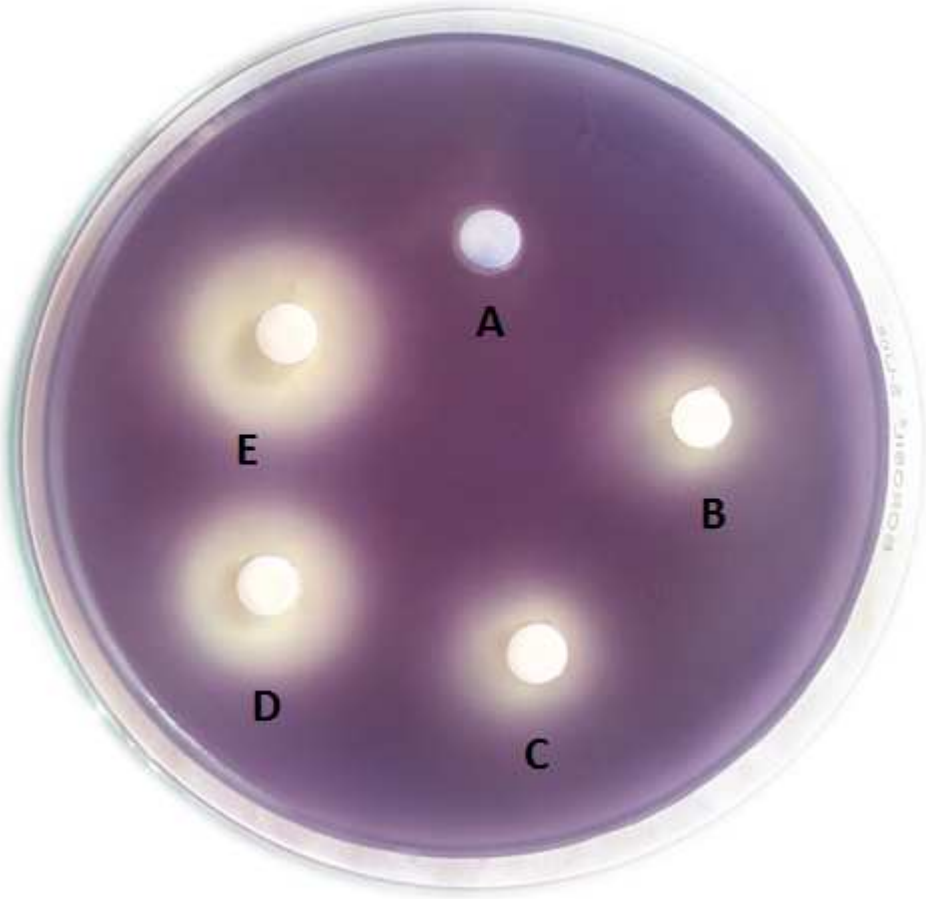
Concentration (µg/mL) of antibiotics were given in paranthesis.

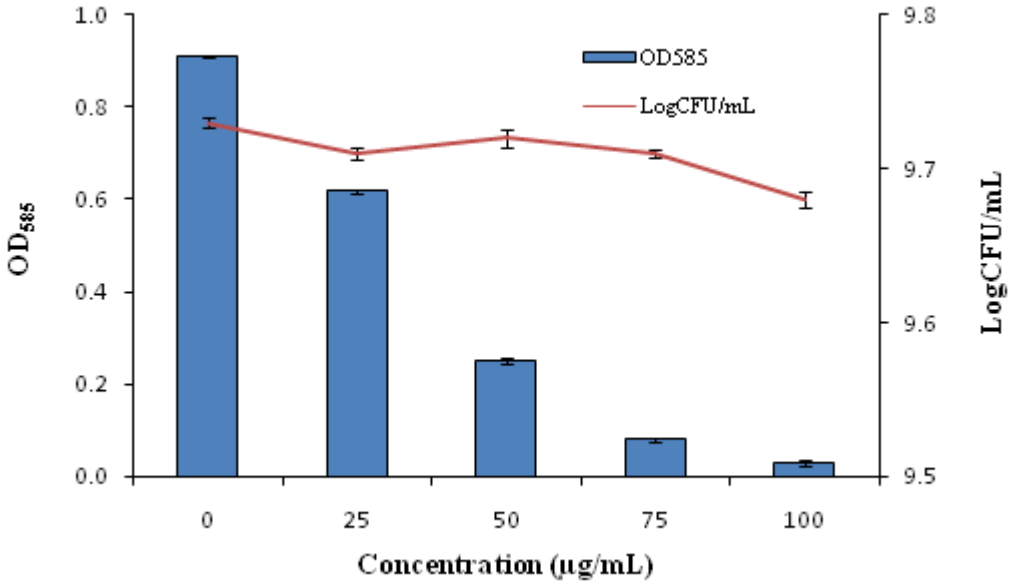
Table 2: *In silico* docking score of resveratrol, an active constituent of Resveramax, with LasR receptor protein from *P. aeruginosa* PAO1.

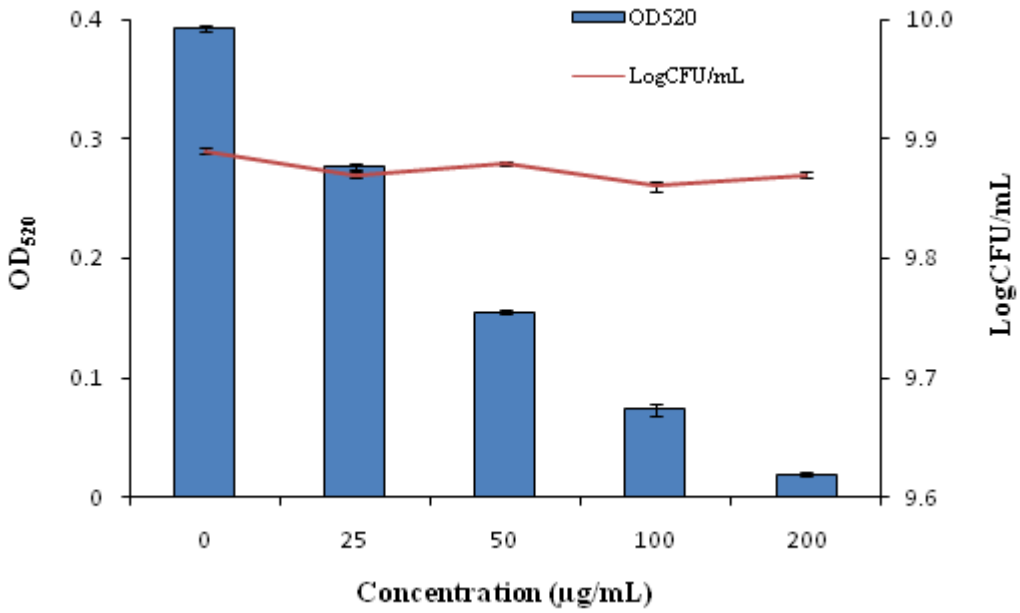
| Binding energy (kJ/mol) | Ligand efficiency (kJ/mol) | Inhibitory constant (µM) | H-Bond | Interaction |
|---|---|---|---|---|
| -8.55 | -0.5 | 542.74 | 3 | Asp73<br>Thr75<br>Leu125 |

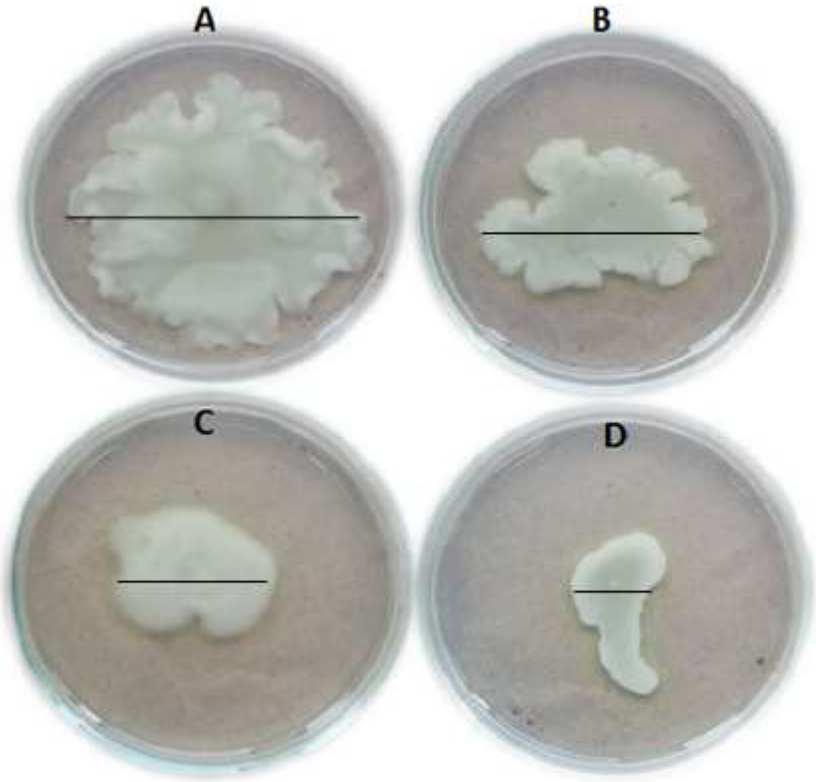
**Supplementary material**

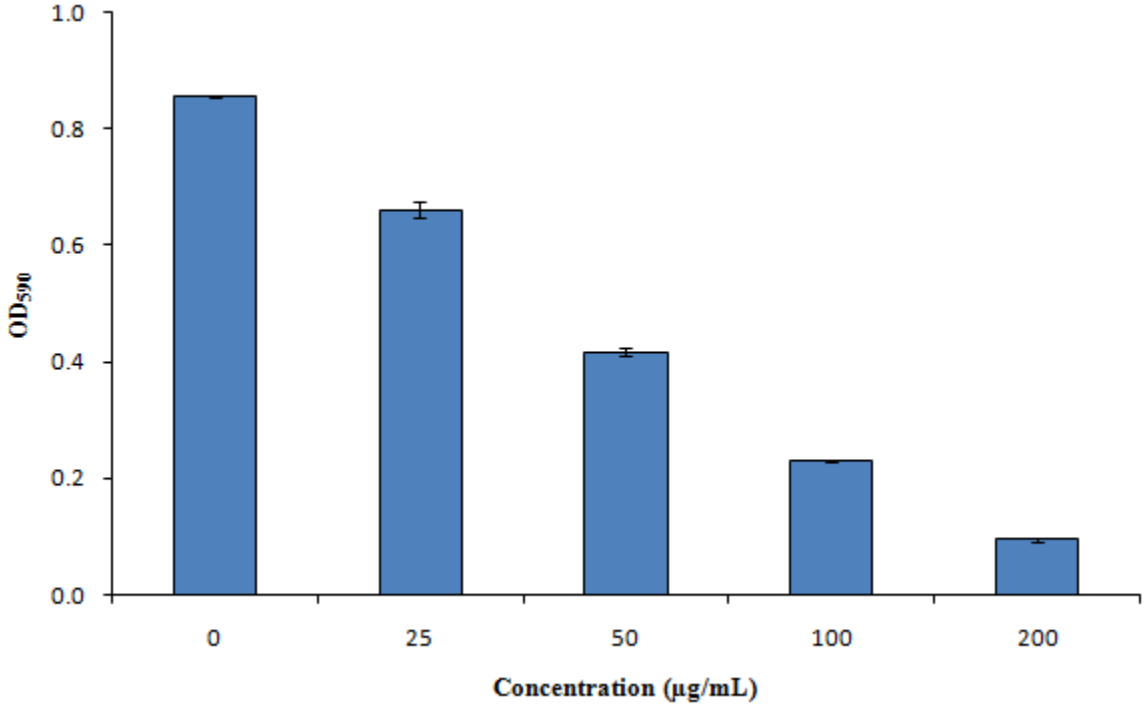
Fig. S1: Anti-QS activity of Resveramax and resveratrol against *C. violaceum* 12472. (A) Resveramax (100 µg/disc); (B) resveratrol (100 µg/disc); (C) vehicle control (1% sunflower lecithin); (D) vehicle control (1% grape seed oil).

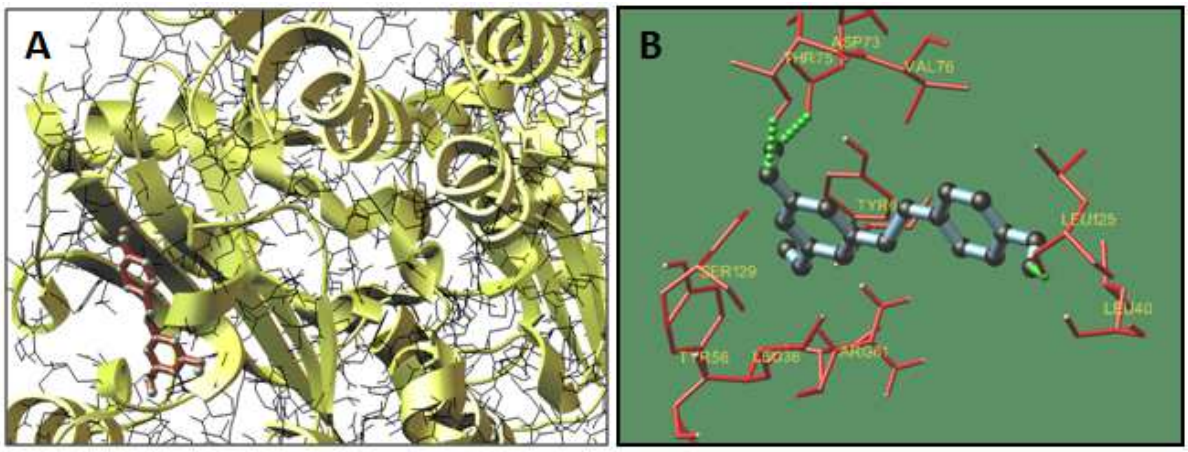












## Highlights

- Interfering with quorum sensing (QS) mechanism in bacteria by plant-derived compounds offers an attractive strategy to combat bacterial infections.
- Bioavailability-enhanced Resveramax$^{TM}$, a novel formulation of resveratrol, inhibited QS in *Chromobacterium violaceum* 12472 and *Pseudomonas aeruginosa* PAO1.
- Biofilms treated with Resveramax showed increased susceptibility to antibiotics.
- Molecular docking analysis proved that resveratrol binds more rigidly with LasR receptor protein with docking score of -8.55 KJ/mol.