

## Anti-Quorum Sensing Potential of Antioxidant Quercetin and Resveratrol

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### ABSTRACT

Quorum sensing system plays an active role in the regulation of pathogenicity of many microorganisms. Inhibition of pathogenicity or virulence factors will increase the success of treatment by preventing the development of antibiotic resistance. In this study, anti-quorum sensing activities of quercetin and resveratrol compounds, which have antioxidant property without damaging to host, have been determined via using biosensor bacteria: *Chromobacterium violaceum* ATCC 12472 and *Chromobacterium violaceum* CV026. As quorum sensing inhibitors, quercetin and resveratrol's cutting off the bacterial communication will prevent the treatment failures caused by the development of bacterial resistance. The development of layered drugs with antioxidant compounds such as quercetin and resveratrol will pave the way for new horizons for new therapeutic strategies.

**Key words:** Anti-quorum sensing, Resveratrol, Quercetin, Antioxidant



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## INTRODUCTION

Many civilizations have used plants for treatment purposes throughout the history of civilizations. The methods of herbal treatment written on the papyrus in the mummies tomb of ancient Egyptian civilizations have been replaced by tablets in Hittite period and have come up to today as written sources<sup>1</sup>. The implementations of herbal treatment have also been come across in the tablets of Roman Empire dominating Europe and Asia Minor. Herbal therapy has not only been used in ancient times but also come up to today and it still continues to be used<sup>1</sup>. Benefiting from plant extracts particularly for the treatment of pathogens, has also contributed to the development of pharmaceutical sciences. Besides being commonly found in nature, plants can also be used as food sources by living bodies<sup>18</sup>. The toxic effects of the plants that are used have enabled them to be preferable for many antimicrobial researches.

As a result of using antimicrobial efficacy molecule for the elimination of pathogens, microorganisms have developed resistance to antimicrobial agents via developing different mechanisms<sup>1, 2</sup>. Therefore, narrowing the areas of many antibiotics used for treatment has led to the development of new antimicrobial agents<sup>2, 7</sup>. Plant extracts have pioneered among the new resources. No matter how efficient the plant extracts have been identified for treatment, it has also been observed to damage to living bodies when they are not used appropriately<sup>7</sup>. In many studies conducted, the compounds with the lowest damage to living bodies have been preferred and the compounds with antioxidant properties, which are popular topics of our age, have been focused on. Some molecules with antioxidant properties are known to be used as antimicrobial agents<sup>1, 3, 1</sup>. Decreasing effectiveness of antimicrobial agents on the virulence of the bacteria, has led to researches to be conducted on bacterial communication system (quorum sensing) which is responsible for the organization of this mechanism<sup>2, 1</sup>. Besides elucidating the quorum sensing, the prevention of this system will ensure success against the bacterial virulence<sup>11</sup>.

Various plant extracts are used in the inhibition of bacterial communication system which is also referred as anti-quorum sensing<sup>3</sup>. The content of these extracts consists of a variety of compounds. How these compounds are effective on living bodies have not been elucidated in researches. In our study, quercetin and resveratrol compounds, whose antioxidant properties are known, have been used. Quercetin is the yellow pigment which is commonly found in the pericarp within the flavonoid group such as apple, onion, tea, and red grape<sup>12</sup>. Anti-carcinogenic, anti-inflammatory, and anti-histamine effects of quercetin have been identified in several studies<sup>4</sup>. Resveratrol is a natural phytoalexin found in particularly red grapes, pineapple, and peanuts<sup>1</sup>. In addition to protecting plants from UV rays, it also benefits in protection against some pathogens. For example, resveratrol, under adverse weather conditions, is produced by black grape against fungal factors. When compared to many other substances, resveratrol is a molecule with high antioxidant properties. Besides anti-aging effects, its effect of lowering blood fat has been determined<sup>4</sup>.

## MATERIAL AND METHODS

### Determining Anti-Quorum Sensing Activity of Quercetin and Resveratrol Molecule

Quercetin (Sigma Aldrich), which has the properties of a natural antioxidant compound and different concentrations of resveratrol (Sigma Aldrich) have been used to observe anti-quorum sensing activity in this study. Quercetin is dissolved in

dimethyl sulfoxide (DMSO) and resveratrol in ethanol. As C6-AHL (N -Hexanoyl-L-homoserine lactone) molecule is frequently used by Gram negative bacteria as communication molecule, anti-quorum sensing activity experiments have been conducted on this molecule. Obtained from the University of Nottingham, pure N -Hexanoyl-L- homoserine lactone molecule has been prepared as 2mg / ml (ethyl acetate) to be used.

#### **Anti-Quorum Sensing Disc diffusion and Agar Diffusion Test**

After *C. violaceum* biosensor strain culture turbidity produced at 30°C for 18 hours is adjusted to Mc Farland 0.5 ( $10^8$  cfu/ml), 100  $\mu$ l *C. violaceum 026* and 50  $\mu$ l C6-HSL extracts have been added to 10 ml soft Luria Bertani agar medium. Quercetin at different concentrations (2, 5, 10, 50, 100, 150, 200, 250 mg/ml ) or resveratrol impregnated discs have been placed on solidifying soft LB-agar medium and has been left in the incubator at 30°C for 48 hours for incubation. For the agar diffusion test; when LB medium consisting of 0.9% agar, 100  $\mu$ l *C. violaceum 026* and 50 $\mu$ l C6-HSL extract has solidified, 4mm wide wells have opened. Quercetin and resveratrol at different concentrations (2, 5, 10, 50, 100, 150, 200, 250 mg/ml) have been added to these opened wells. Petri dishes have been left for incubation at 30 ° C for 48 hours. Agar diffusion and disc diffusion tests have been repeated three times

12, 10, 3, 4, 1, 11.

#### **Spectrophotometric Measurement of Anti-Quorum Sensing Activity of Quercetin and Resveratrol**

In accordance with the amounts given in Table 1., Quercetin and resveratrol at different concentrations (2, 5, 10, 50, 100, 150, 200, 250 mg/ml ) have been added to eppendorf tubes. The amount of violacein pigment formed after incubation has been measured at 585nm in spectrophotometer and anti-quorum sensing activity has been detected.

#### **Violacein Pigment Isolation**

Extraction of violacein pigment from liquid culture has been done as Blosser and Gray<sup>1</sup>. 100  $\mu$ l from 20 ml of 15-hour fresh culture of *C. violaceum 026* strain produced in LB medium has been taken and cultivated in 1ml LB medium in the framework of procedure given in Table 1. The culture left for incubation at 30°C for 24 hours has been mixed with vortex and 200  $\mu$ l culture has been taken from this tube and transferred to eppendorf tube. 200  $\mu$ l of 10% sodium dodecyl sulfate (SDS) has been added on it for the lysis of the cells and vortexed for 5 seconds and kept for 5 minutes at room temperature. 900  $\mu$ l butanol (as 50ml n-butanol + 10ml distilled water) which is saturated with water for the violacein pigment isolation, has been added to tube and vortexed for 5 seconds. Thereafter, it has been centrifuged for 5 minutes at 10,000 rpm. The upper phase has been transferred into a new tube and read at 585 nm on microplate spectrophotometer and the amount of violacein has been determined<sup>3</sup>.

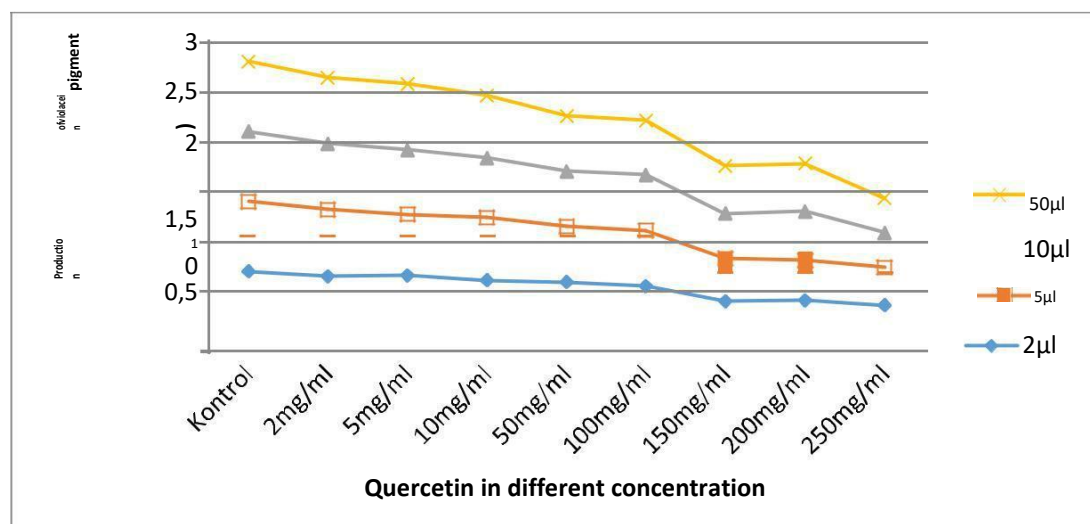
**Table 1.** Quercetin and resveratrol Application Procedure

	A	B	C	Final concentration
Luria Bertani Broth	895 µl	890µl	890µl	
CV026	100 µl	100µl	100µl	$1 \times 10^8$ cfu/ml
C6-AHL	50 µl	50 µl	50 µl	150µmol/ml
Quercetin	-	-	5 µl	Different concentration of
Resveratrol	-	-	5 µl	Resveratrol or quercetin (2,5,10,50,100,150,200,250 mg/ml )
DMSO or Etanol	-	5 µl	-	
Total volume	1045 µl	1045 µl	1045 µl	

## RESULTS AND DISCUSSION

### Anti-Quorum Sensing Activity of Quercetin Molecule

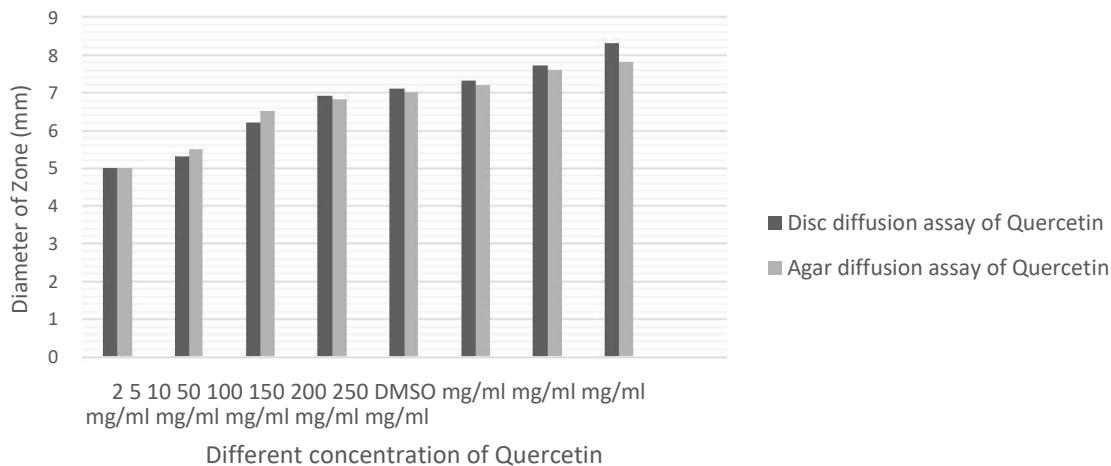
As mentioned, 2, 5, 10, 50, 100, 150, 200, 250 mg/ml concentrations of Quercetin have been prepared by dissolving in DMSO. As a result of the study, it has been determined that Quercetin with antioxidant properties has had positive impact on living bodies and C6-HSL which is the bacterial communication molecule. As a result of both agar diffusion test and disc diffusion test, different concentrations of quercetin have been observed to inhibit violacein pigment production (Figure 1 and Figure 2).

**Figure 1.** Anti-Quorum Sensing Activity of Quercetin (50mg/ml)**Figure 2.** The effect of different concentrations of resveratrol on violacein pigment synthesis.

As it has been observed that the production of bacteria has been inhibited in the zones formed as a result of both agar diffusion and disc diffusion tests (Figure 3), the amount of violacein pigment has been determined by spectrophotometric means

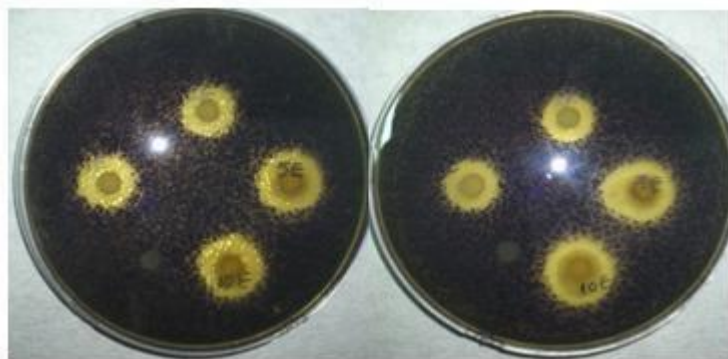
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as a result of formed anti-quorum sensing effect lessening the production of bacteria, and inhibitory effect of quercetin on C6-AHL in addition to studies above.



**Figure 3.** Disc and agar diffusion assay of Quercetin

In this study, the declining production of violacein pigment, in other words quercetin's inhibition of C6-AHL molecule has been determined. *C. violaceum* 026 biosensor strain produces violacein pigment in the presence of C6-AHL, which is a communication molecule, and this production regulates bacterial communication mechanism called quorum sensing. As known, C6-AHL is used as communication molecule by many Gram negative pathogens bacteria (*Pseudomonas aeruginosa*, such as *E. coli*). As shown in Figure 4 the more quercetin concentration increases the more violacein pigment production decreases, in other words quercetin inhibits bacterial communication system.



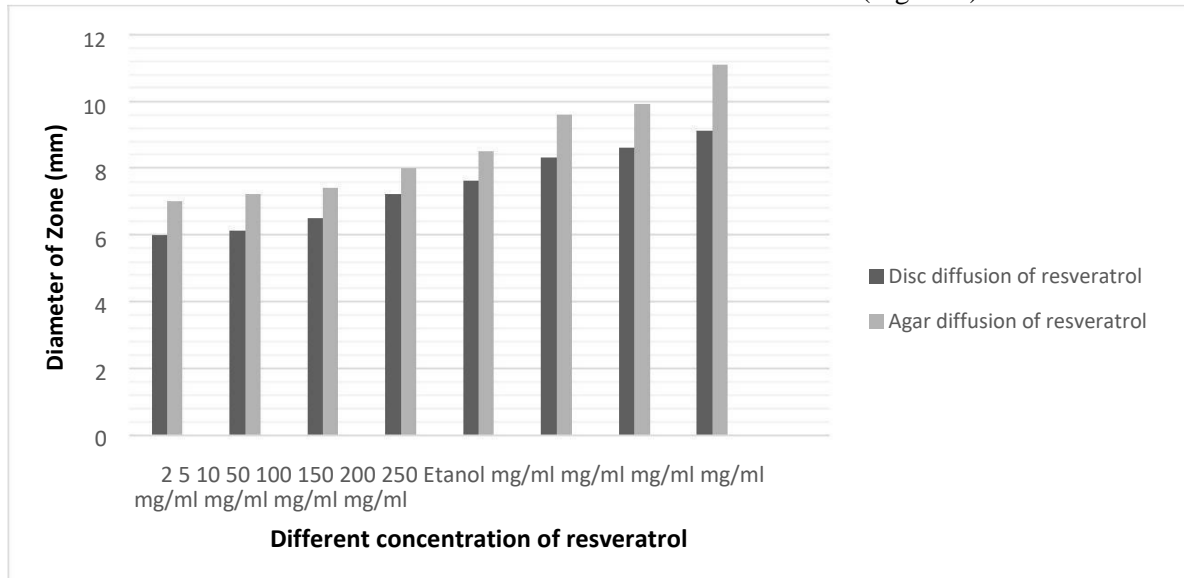
**Figure 4.** Anti-Quorum Sensing Activity of Resveratrol (50mg/ml)

Thus, that the antioxidant properties of a molecule such as quercetin inhibit bacterial communication paves the way for a new dimension and hope in antibacterial studies. Pathogenic bacteria's gaining resistance against antibacterial agents leads to misapplication of treatment, thereby the extension of the treatment process, and huge economic losses. Also, antibacterial agents in living bodies have huge side effects. Thanks to studies conducted for the cessation of bacterial communication, bacteria will not be able to develop resistance to molecules used and therefore the possibilities of treatment will increase for the patients. This research shows that anti-quorum sensing activity of quercetin molecule with antioxidant properties is in

question and bacterial communication molecules which are frequently used by Gram-negative pathogenic bacteria are inhibited in this way.

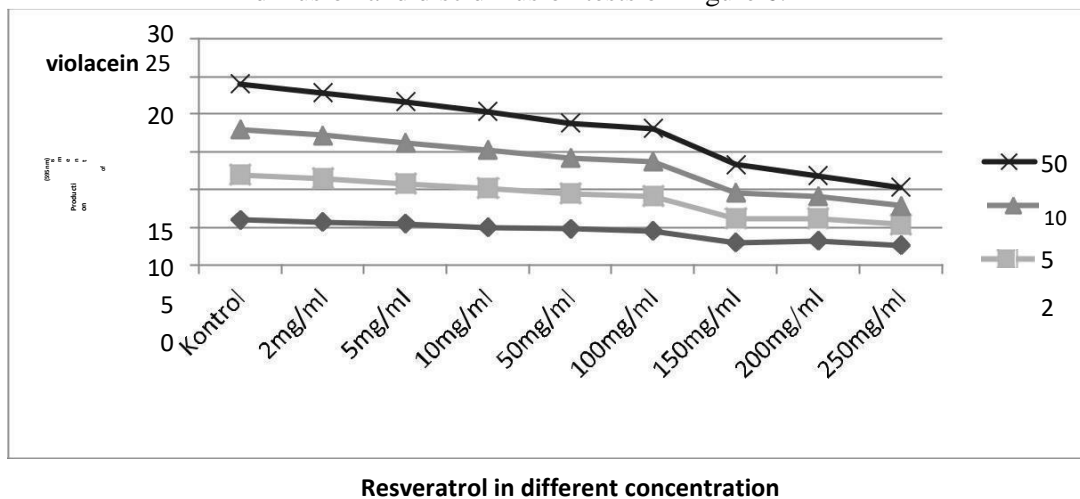
### Anti-Quorum Sensing Activity of Resveratrol Molecule

As mentioned, 2, 5, 10, 50, 100, 150, 200, 250 mg/ml concentrations of resveratrol have been prepared by dissolving in dimethyl sulfoxide (DMSO). For Anti-quorum sensing activity studies, disc diffusion and agar diffusion tests have been conducted and diameters of zones have been measured as mm (Figure 5).



**Figure 5:** Disc and agar diffusion assay of Resveratrol

Resveratrol is in the forefront among the antioxidant molecules which have positive impact on health. Due to its strong antioxidant properties, it protects the cells from the damage of free radicals. Resveratrol may decrease the effects of certain fungi and bacteria. Based on this property, the efficacy of resveratrol on bacterial communication system has also been analyzed. Anti-quorum sensing activity of resveratrol has been identified on C6-AHL molecule which is commonly used by Gram-negative bacteria species for communication. It has been observed that the more concentration of resveratrol used in different concentration has increased, the more its efficacy on signal molecule has increased. In majority of anti-quorum sensing activity studies, violacein pigment isolation has been conducted besides agar diffusion and disc diffusion tests on Figure 6.



**Figure 6.** The effect of different concentrations of resveratrol on violacein pigment synthesis.

The purpose of this is to understand whether the formed effect is antibacterial or it is because of the inhibition of signal molecule. As a result of our study, it has been determined that resveratrol, which is an antioxidant compound, is also effective on bacterial communication system. Anti-quorum sensing activity study is an applicable strategy against the resistance that bacteria developed against antibacterial agents. Kerekes et al., have studied the effects of essential oils extracted from sage, juniper, lemon, and marjoram on biofilm produced by bacteria and yeast and C6-AHL which is a bacterial communication system molecule<sup>4</sup>. As a result of the study, these essential oils have been found as good candidates to inhibit AHL-mediated QS mechanism. Essential oils are known to possess antimicrobial effects. It could be argued that this effect occurs by the way of inhibiting signaling molecules. Adonizio et al, have studied anti-quorum sensing activities of 50 plants with high medical importance isolated in South Florida and determined that 6 plant species have high anti-quorum sensing activity<sup>3</sup>. Alvarez et al., have determined the efficacy of tea tree and propolis on violacein pigment and proposed that these compounds have anti-quorum sensing activity<sup>3</sup>. Similar to this study, Priya et al., have tried the methanolic extracts taken from *Phyllanthus amarus* Schumac & Thonn plant known as Chinese herb on *Escherichia coli* (*E. coli*) [pSB401], *E. coli* [pSB1075] and *C. violaceum* CVO26 sensor bacteria and determined that the signaling molecules in the medium have been inhibited<sup>11</sup>. Another study about showing the effects of plant extracts and all on quorum sensing mechanism has been conducted by Chu et al and they have proved that Rhubarb, Fructus Gardeniae, and *Andrographis paniculata* plants have showed anti-quorum sensing activity via using the *C.violaceum* CV12472 and *Pseudomonas aeruginosa* PA01.

## CONCLUSIONS

As it is seen in many of the mentioned studies above, in several plants interwoven with pathogens, some molecules are produced and the effects of bacteria are reduced. These features developed by plants are not just limited to antibacterial effect but also they cut off the communication between bacteria and inhibit their virulence properties. When the conducted studies are analyzed, it is seen that there various compounds in the plant extracts used by the researchers, therefore, which molecule is responsible for anti-quorum sensing activity cannot be explained. In our study, it has been determined that quercetin and resveratrol compounds acquired as pure are efficient in bacterial communication. It has also been determined that quercetin and resveratrol, which gets the attention with their antioxidant properties, can be used in bacterial communication system inhibition, therefore they are the molecules which can replace antibacterial agents without damaging the host.

## ACKNOWLEDGEMENTS

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