An Evaluation of the Anti-QS Activity and Virulence Factors Production Potential of Rhamnus cathartica L. against Some Gram-Positive and Gram-Negative Bacteria

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Article

An Evaluation of the Anti-QS Activity and Virulence Factors Production Potential of *Rhamnus cathartica* L. against Some Gram-Positive and Gram-Negative Bacteria

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Abstract: The study aims to evaluate the Quorum Sensing (QS) system inhibition against some Gram-positive and Gram-negative bacteria detected by molecular modeling of *R. cathartica* L. plant extract. *R. cathartica* L. methanol extract was evaluated in vitro. The research was carried out for the possible role and mechanism of *R. cathartica* L. The antibacterial activity of *R. cathartica* L. seed extract on some Gram-positive and Gram-negative strains was evaluated using culture-based and computational analysis. Gentamicin (CN; 30 µg/ml, Bioanalyse) was used as an antibacterial control of the extract on the production potential of QS-dependent virulence factors on *Pseudomonas aeruginosa* PAO1 was investigated. Phytochemical analysis resulted in the identification of 23 phenolic phytochemicals. *R. cathartica* L. extract was observed to have antimicrobial activity against Gram-positive and Gram-negative bacteria at different rates (11.3 mm-16 mm). The inhibitory effect of *R. cathartica* L. extract on *P. aeruginosa* PAO1 (elastase 61%, pyocyanin 18%, and biofilm 61%) was recorded moderately. Phytochemical analysis revealed that the main component of the extract is kaempferol. Therefore, the binding potential of kaempferol on QS system receptors was investigated via molecular docking. Computational analysis showed that kaempferol can inhibit the QS system by preventing competitive ligands from connecting to LasR. This study explored the activity and mechanism of action for a new green alternative against resistance. might provide a mechanism for *R. cathartica* L. seed extract components and thus provide a new avenue for using *R. cathartica* L. seed extract as a green antibacterial agent.

Keywords: quorum sensing; molecular docking; phytochemical; *Rhamnus Cathartica* L; plant-based antimicrobial

1. Introduction

In recent years, one of the biggest problems on the global health agenda has been antibiotic resistance. Its effects on public health and the economy have been among the topics worldwide Especially recently, the rapid antibiotic resistance rates have sustainable development, the global economy, trade, and the stability of countries, and the predictions that it will have effects in the coming years are increasing [1,2]. Among these infectious-causing bacteria, *P. aeruginosa* is a lethal bacterium, especially in immunosuppressive patients. The biggest problem associated with antimicrobial treatment failure is the emergence of resistance. Furthermore, *P. aeruginosa* cells communicate via QS by synthesizing small signaling molecules that associate the regulation of virulence factors such as elastase, pyocyanin, biofilm. It is known that the missuse of antibiotics and

the transfer of bacteria-resistance genes lead to difficulties. [3]. In this context, the treatment of many diseases using plants has been a frequently used solution to fight against microorganisms and was seen as a promising strategy [4]. In addition, due to the richness of the chemical structures of plants, their use in the development of highly effective drug formulations has been one of the research areas of pharmacology. Bioactive compounds that develop the secondary metabolic activities of plants and cannot be consumed as food but have beneficial effects on human health are called 'phytochemicals'. The best-known phytochemical compounds are phenolic compounds (polyphenols), tannins, indoles, saponins, carotenoids, tocopherols, coumarins, terpenes, isothiocyanates, sulfites, terpenoids, sulforaphane, flavonoids, phytosterols and phytoestrogens. [5] These compounds are rich in phytochemicals with antimicrobial and antioxidant activity, which are used against many diseases today [6] Flavonoids, which represent an important group of phenolics, are secondary metabolites with significant antioxidant, antimicrobial activity, and chelating properties [5] In the literature, antioxidant of flavonoid compounds [7] anti-inflammatory [8] antiallergic, antiviral [9] and anticarcinogen (cytotoxic) [10] a wide variety of bioactive activities have been reported. The main component of the flavonoids group is flavones, and the most important members are routine, apigenin, epicatechin, kaempferol, eriodictiol, fisetin, and luteolin have been reported [11]. Kaempferol has been shown to prevent the growth of bacteria and increase antimicrobial activity by inhibiting the efflux pumps of resistant *S. aureus* [12] Additionally, studies that kaempferol *E. coli* [13]. *Bacillus spp*. [14] *Acinetobacter baumannii* [13] *K. pneumoniae* [15] *M tuberculosis* [16], *P. aeruginosa* (Shu-Chen et al. 2020), *Salmonella spp*. [15], *Enterococcus faecalis* [18] and *P. vulgaris* [15] are effective against different types of bacteria. *R. cathartica L.* (Buckthorn) belongs to the *Rhamnaceae* and is used as medicine among the subtropical regions of North and South America, East Asia, and Africa [19]. The *Rhamnaceae* family has a worldwide distribution but is more common in tropical and subtropical regions. While *Rhamnus* is represented by about 100 species worldwide, 22 have a natural presence in Turkey, and 6 are endemic. Species belonging to this genus, in which endemic species mostly spread around the Mediterranean, are shrubs with different heights ranging from short shrubs that can grow up to 0.5 m to 4-5 m in height [20]. Previous studies highlighted the antimicrobial effect of the herbal extract of other components of the *Rhamnus* [19,21]. Previous studies revealed the molecular sequences of the plant-derived agents used in the treatment, researching and formulating the usage doses, determining the safety and effect of use, and even pharmacological profiles have led to the expansion of the field of ethnopharmacology. A study investigating the antibacterial activity of Kaempferol showed apoptosis and DNA fragmentation following disruption of the *Micrococcus luteus* cell membrane. Studies have shown that Kaempferol damages the cell membrane of *E coli* and inhibits cell function and biofilm formation against *Pseudomonas* aeruginosa, Mycobacterium and *Vibrio cholerae* [22–24] The kaempferol is the most potent flavonoid at directly blocking bacterial DNA gyrase, which is another significant antibacterial mechanism on *E. coli* [25]

Kaempferol has also been shown to inhibit *S. aureus* DNA helicases, allowing it to be used as lead compounds in new antibiotics. Therefore, with the increase in antibiotic resistance natural compounds may offer a promising option as they directly or indirectly affect the biochemical processes of cells and disrupt their physicochemical integrity. As a result of the literature review, it was aimed to investigate the antibacterial activity of the extract prepared from the *R. cathartica* L. plant on bacteria and its inhibition effect on the QS system. In our study, the mechanism of action of QS system inhibition was elucidated by molecular modeling.

2. Materials and methods

2.1. Plant material and extract preparation

R. cathartica L. seed was collected from the Alay district of Osh province in Southern Kyrgyzstan and supplied commercially. First, the samples were powdered with a blender (Waring 8011 EB, USA). Briefly, 8.8 g of powder material was weighed 88 mL of solvent (methanol) with extracted in an ultrasonic bath (Elmasonic P, Germany) for 45 min. After the solvent-powder mixture was extracted in an ultrasonic bath for 15 minutes, it was filtered with coarse filter paper and the solvent was

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completely removed in a rotary evaporator (Heidolph Hei-Vap Rotary Evaporator) under vacuum at 40-45ºC. After the solvent had completely evaporated, the plant extract was dissolved with Dimethylsulfoxide (DMSO) and stored in freezer until experiments.

2.2. Biochemical contents analysis

The extract prepared with *R. cathartica* L. was analyzed by HPLC (High-Pressure Liquid Chromatography) in triplicate using an HPLC device (RP-HPLC, Shimadzu Scientific Instruments, Tokyo, Japan). A 90 min gradient program was applied to separate and detect quercetin, luteolin, kaempferol, apigenin, gallic acid, protocatechin acid, p-hydroxybenzoic acid, catechin, caffeic acid, epicatechin, syringic acid, chlorogenic acid, vanillin, *p*-coumaric acid, benzoic acid, ferulic acid, sinapinic acid, *o*- coumaric acid, rutin, hesperidin, eriodyctiol, rosmarinic acid, and cinnamic acid. Operating conditions and gradient programs are given in Table 1. Solutions were prepared and an ultrasonic bath was used to remove air bubbles. After the gradient program was finished, the mobile phase was passed through the column for 10 minutes to equilibrate.

2.3. Screening of seed extract for antibacterial activity

R. cathartica L. seed extract was tested for antibacterial activity against Gram-positive (*Enterococcus faecalis* ATCC 29212, Methicillin-Resistant *Staphylococcus aureus* (MRSA), ATCC 43300, *Staphylococcus aureus* ATCC 25923) and Gram-negative (*Pseudomonas aeruginosa* PAO1; *Escherichia coli* ATCC 25922) standard strains. Antibacterial effect of extract was determined by the agar well diffusion method. Microorganisms were inoculated to the medium "Luria-Bertani" (LB) and incubated at 37 °C overnight to ensure their activation. The overnight cultures was standardized with the 0.5 McFarland standard and 100 µl inoculated into Petri dishes containing Mueller Hinton Agar (MHA). Using a sterile loop, 6 mm diameter wells were drilled in the solid medium, and these wells were then filled with 100 µl seed extract. Petri dishes were incubated, and the inhibition zone around the wells in each petri dish was measured. Gentamicin CN (30 µg/mL, Bioanalyse) was used as a control to test the susceptibility of bacterial strains [26].

2.4. Screening of seed extract for Qs inhibitor activity

2.4.1. Performing the biofilm test

The biofilm inhibition effect of seed extract on *P. aeruginosa* PAO1 was investigated crystal violet (cv) method [27,28]. A twenty µL of extract and 10 µL of PAO1 bacterial culture equivalent to 0.5 McFarland turbidity were transferred to 96-well plates containing 200 µL of LBB medium and incubated for 24 h. After the incubation phase, the plate content was poured out and washed 3 times with distilled water. Then 200 µL of 0.1% cv solution was added to the wells and waited 30 min. The next step consisted of decanting the crystal violet and washing the plate 3 times with distilled water. Finally, 200 µL 95% ethanol was transferred to the wells and waited for 15 minutes. Results were read at 570 nm. (BioTek Epoch Microplate Spectrophotometer, USA) (O'Toole., 2010). The results were evaluated by comparison with the *P. aeruginosa* PAO1 strain, which has biofilm-producing properties. The reference PAO1 was used as a positive control. Sterile LBB was kept as a negative control. The inhibition rate of biofilm formation was determined following the formula.

Inhibition rate (%) = [(OD in control –OD in sample) × 100] /OD in control

OD (Optical Density at 570 nm=0.05)

2.4.2. Performing the elastolytic test

The elastolytic activity of the extract was determined according to the method of Fuentes [30]. The inhibitory effect of extracted oils on elastase production was investigated by the Elastin Congo Red (ECR) test. 100 µL of plant extract was added to each well and incubated at the incubator shaker at 37°C for 14-16 hours. It was transferred from the supernatant of the culture to a 100 µl tube and 900 µl ECR buffer. (100 mM Tris, 1 mM CaCl2, Ph 7.5) was added. The final mixture was incubated

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at 200 rpm for 3 hours at 37°C. Centrifugation and removal of the undissolved ECR as a result of incubation and reading the supernatant at OD 495 nm were also the last steps of the method, and the study was carried out in 3 replications.

2.4.3. Performing the pyocyanin test

The effect of the extract on pyocyanin pigment production was determined as described by Fuentes. To investigate the inhibitory effect of *R. cathartica* L. methanol extract on pyocyanin pigment production; inoculated previously with *P. aeruginosa* pyocyanin pigment production was added to 100 µL of LBB medium; OD 0.05 at 570nm was added along with the bacterial culture and incubated at 37°C for 16-18 hours with shaking. Then, 5 mL of chloroform was added to the culture medium and vortexed for 30 seconds. The separated phase was placed in 2 mL glass tubes and 1 mL of HCL-H_2O mixture (0.2 mmol-1) was transferred onto it. The absorbance of the pink phase formed at the top of the tubes at the end of the vertexing process was recorded at 520 nm.

2.5. Molecular docking

Three-dimensional (3D) structures of LasR with PDB (protein data bank) code of 2UV0 [31]. and PqsR with PDB code of 7NBW [32] were retrieved from PDB. Ligands were retrieved from PubChem [33]. After the water molecules were deleted from the structure of the receptors, the proteins and ligands were prepared for molecular docking by adding polar hydrogens and assigning the Gasteiger charge. Molecular docking was performed using the AutoDock Vina program [34]. The molecular docking data were visualized and analysed with the Biovia Discovery Studio program [35].

2.6. Statistical analysis

The data were obtained by performing three repetitive experiments according to the random parcel design, and JMP 8 package statistics program was used for the analysis. Differences between means were evaluated with the LSD (Least Significant Difference) multiple comparison test.

3. Results

3.1. HPLC analysis of compounds

According to the HPLC analysis results of R. cathartica L. extracts, phenolic acid compounds (gallic acid, protocatechuic acid, p-hydroxybenzoic acid, caffeic acid, p-coumaric acid, ferulic acid, o-coumaric acid, quercetin) and flavonoid compounds (catechin and kaempferol) were identified. However, among the tested standard compounds, chlorogenic acid, epicatechin, syringic acid, vanillin, sinapinic acid, benzoic acid, rutin, hesperidin, rosmarinic acid, eriodictiol, cinnamic acid, luteolin, and apigenin were not detected. Among the detected compounds, kaempferol showed the highest concentration (781.7 µg/g), catechin, quercetin, and protocatechuic acid, was detected at 677.6 µg/g, 313.1µg/g, and 189.4 µg/g, respectively. The phenolic compounds detected in the study and their concentrations are given in Table 2. The chromatogram showing the separation of the phenolic compound in the column is shown in Figure 1.

3.2. Antibacterial activity

The data obtained showed different results on bacteria. According to the results obtained, it was observed that *R. cathartica* L. extract had antimicrobial activity at different rates (11.3 mm-16 mm) against Gram-positive bacteria. *R. cathartica* L. extract had high antimicrobial activity on Gram-positive bacteria *B. cereus*. Among Gram-negative strains, *R. cathartica* L. extract was found to have high antimicrobial activity on *E. coli*. The results were statistically significant ($p<0.01$) Figures 2 and 3.

3.3. Confirmation of the anti-QS activity

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According to the bacterial test results, *R. cathartica* L. methanol extract was found to have an inhibitory effect against three factors that have an important role in *P. aeruginosa* virulence. The inhibitory effect of the extract on biofilm and pyocyanin formation was 61%, and the effect on elastase formation was 18% Figure 4.

3.4. Molecular docking

The phytochemical analysis revealed that kaempferol and catechin are the most abundant constituents of *R. cathartica* L. extract. Moreover, the extract was found to be active against the QS system. Hence, molecular docking of kaempferol with QS system receptors (LasR and PqsR) was conducted in this study to elucidate the extract's mechanism of action. Kaempferol had a good binding with LasR (Figure 5a, Table 3). The binding mode of kaempferol was better than its natural ligand, OdDHL (N-3-Oxo-Dodecanoyl-L-Homoserine Lactone) (Figure 5b, Table 3). The binding energy of kaempferol and OdDHL were -9.3 kcal/mol and -5.7 kcal/mol, respectively. This has alos implicated that kaemferol could have higher affinity toward LasR relative to OdDHL. The binding mode of kaempferol with PqsR was also not bad (Figure 5c). There was no common interaction residue with its standard ligand, NHQ (2-nonyl-4-hydroxyquinoline) (Figure 5c,d). The binding energy of kaempferol and NHQ was -6.9 kcal/mol and -7.2 kcal/mol, respectively. The binding energy of kaempferol and NHQ had a slim difference that implicated similar affinity toward PqsR. Together with this, kaempferol formed two conventional hydrogen bonds with PqsR whereas NHQ had null (Figure 5, Table 3). The higher number of hydrogen bonding could bring a stronger interaction to the target but the number of other interactions for NHQ was much higher than that of kaempferol (Table 3). Therefore, the binding energy and strength obtained implied that the two ligands might have similar interaction potential to PqsR. The molecular docking analysis exhibited that the binding affinity of kaempferol with LasR was higher than that of PqsR.

3.5. Discussion

Many types of microorganisms exhibit social behaviour. They communicate with each other through the signal molecules they have produced, monitor whether they have reached an absolute majority, and trigger critical gene expressions such as the synthesis of virulence factors as soon as they get a sufficient majority. It is known that N-acyl-homoserine lactone is an autoinducing molecule known as AHLs and forms the QS mechanism in Gram-negative bacteria. It is stated that Als is also responsible for the regulation of functions in different biological domains, such as the expression of some genes of plant pathogenic microorganisms, *Pseudomonas*. It is reported that some species of *Bacillus* provide enzymatic inactivation on Als. In this case, it is thought that the use of another microbial signaling molecule may be appropriate in terms of inducing or inactivating microbial activity. Thus, it creates a successful infection process by not stimulating the host's immune system prematurely [36,37]. As a result, the problem of resistance to antibiotics, which is the most significant barrier in the fight against infections, arises [38] Plants, which are frequently used in traditional medicine systems, are also actively used in the modern pharmaceutical industry, and with known active ingredients, they are being used against several disease conditions. Flavonoids, secondary metabolites, are widely available in the plant kingdom, both in free form and the form of glycosides, with a wide range of pharmacological [38] Derivatives of various flavonoids from the genus Rhamnus have been reported. Members of this genus have had a wide range of applications in modern medicine from ancient times to the present [39] In addition, the plants of the genus *Rhamnus* are known for antimicrobial, antirheumatic, hypoglycemic, anthelmintic, antipyretic, antiepileptic, and antidiabetic, in the treatment of vertigo, headache, eye diseases, and elimination of intestinal worms [19] . The main strategy to avoid or reduce QS-regulated bacterial virulence is to block the receptor by structural analogs of the QS signaling molecule. Flavonoids have been found to suppress the virulence of bacteria through allosteric inhibition of the LuxR-receptor [40].

Phenolic acids and flavonoids are found in *R. cathartica* L. extract. In addition, apigenin, kaempferol, quercetin, kaempferol-3-O-isoramninoside, rhamnocitrine-3-O-isoramninoside and rhamnetin-3-O-isoramninoside have been reported from Rhamnus spp extract. Among these,

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kaempferol is found in higher concentrations as reported in previous studies [41] It is known that kaempferol (the highest concentration in *R. cathartica* L. extract) inhibits bacterial resistance by preventing nucleic acid synthesis, energy metabolism, etc., and exerts antimicrobial activity [42]. In a study, kaempferol has been shown to inhibit the formation of bacterial biofilms by disrupting cell walls [43]. *P. aeruginosa* infection is one of the deadly infections caused by the contribution of various bacterial virulence factors, mainly biofilm, and weakening of the host's immune system [44].

Recent studies have revealed that bacteria in biofilms are 1000 times more resistant to antibiotics. Thus, microorganisms in a biofilm have a greater chance of survival and growth in adverse environmental conditions [45]. *Pseudomonas species* can form different pigment molecules. The production of these molecules, known to have various roles in virulence and vital functions, is controlled by two other regulatory systems. These include a two-component transcriptional regulatory system and a QS system. These two mechanisms are essential for the survival and reproduction of the microorganism in the host [2]. Pyocyanin, the blue pigment metabolite of *P. aeruginosa*, plays a significant role in pathogenesis by stimulating and suppressing the host response [46] This pigment also impairs the functions of the ciliated airway epithelium and increases tissue damage by causing the secretion of toxic free radicals. Additionally, pyocyanin deactivates α1-antitrypsin, which adds to the cystic fibrosis protease-antiprotease imbalance [44]. *P. aeruginosa* also binds with its elastase to many extracellular matrix proteins, including fibrinogen, collagen, and elastin, thus contributing to bacterial invasion into the lung parenchyma. Today, various studies have shown that the QS system plays an important role in biofilm formation and increases. Investigating the effects of extracts or extracted oils from plants on the environmental sensing system of important human pathogens will make important contributions to the development of new strategies for the treatment of diseases caused by these pathogens. For this reason, studies on the inhibition of the QS system contribute to the realization of an effective treatment process by reducing the antibiotic tolerance caused by the biofilm [47,48].

In our study, the inhibition effect on biofilm formation was investigated and it was observed that *R. cathartica* L. extract (61%) had an inhibitory effect. Furthermore, among the phytoconstituents of the extract, kaempferol was found to be the most abundant. Since the phytochemical analysis revealed, kaempferol is the major component of the *R. cathartica* L. extract, the anti-quorum sensing activity might result from the inhibitory effect of kaempferol on QS system receptors. Therefore, the potential of kaempferol to inhibit LasR and PqsR was investigated through molecular docking. The outcomes of the computational analysis demonstrated that kaempferol had a high affinity for binding to LasR. Kaempferol was found to bind with LasR with 4 hydrogen bonds (Arg61, Thr75, Leu125, Ser129), pi-anion (Asp73), pi-sigma (Leu36), pi-pi (Tyr64, Gly126), and pi-alkyl (Val76, Ala127) interactions. Furthermore, Kaempferol and OdDHL had common hydrogen bonds at Arg61 (Figure 4, Table 3). In addition to this, crystal structure analysis of the binding of LasR with its autoinducer (OdDHL) in previous study revealed that hydrogen bonds and hydrophobic interactions at some residues played a role in forming the bound structure. Among these residues, there were common hydrogen bonds (Thr75, Arg61, Ser129) and hydrophobic reactions (Asp73, Leu36, Tyr64, Val76, Gly126, Ala127) between kaempferol and OdDHL [31]. Kaempferol had a better interaction potential than OdDHL. The interaction residues of the two ligands were also similar to each other. Moreover, interaction residues obtained from the computational study were compatible to previous experimental studies. In short, these results showed that kaempferol competitively inhibited the binding of OdDHL to LasR, thus preventing its activation of the QS system. Kaempferol was also found to have a good binding affinity with PqsR. However, no common interaction residue with the standard ligand (NHQ) and the X-ray crystal structure of the protein together with the ligand was detected. Furthermore, binding energies of kaempferol and NHQ to form the respective complexes were similar to each other that implied close binding affinity for both. Though there were two conventional hydrogen bonds between kaempferol and PqsR, the number of other interactions for NHQ was much higher (Table 3). Thus, the interaction of the two ligands to PqsR is expected to be similar. Together with this, the absence of common interaction residues for the two ligands decreases the possibility of competitive inhibition. Hence, the potential of kaempferol by inhibiting the QS

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system preventing NHQ from connecting to PqsR is low. If kaempferol had the potential of inhibiting the binding of NHQ competitively, the anti-quorum sensing activity would have been higher. A previous study found that the basic component of cherry extract, in addition to competitively inhibiting LasR, also prevents NHQ from binding to PqsR. In the same study, the QS inhibition level of the cherry extract was higher than the results of other studies[49]. These studies imply that the synergetic effect of major phytochemicals on various receptors in the QS system is crucial to achieving inhibition at a higher percentage. The computational interactions of kaempferol with LasR were like experimentally determined interactions reported previously. Although there was no common interaction point with kaempferol, the computational interaction residues of NHQ with PqsR were observed in an X-ray crystallographic determined interactions of PqsR with a newly synthesized ligand (Ile236, Tyr258) [32]. Thus, the molecular docking results of the reference ligands (OdDHL, NHQ) with their respective receptors (LasR, PqsR) in the computational study validated the docking process, as they were compatible with the experimental structure interaction residues.

In summary, the computational analysis revealed that the QS system inhibition might result from the inhibition of LasR by kaempferol. In addition to this, the synergetic effect exerted by the other components of the extract might have contributed for the observed anti-QS activity.

6. Conclusion

Today, where antibiotic resistance poses a serious health threat, new strategies are sought in the fight against resistant microorganisms, and especially the use of plants and inhibition of the QS system is emphasized. The present study results showed that *R. cathartica* L. extract exhibited antimicrobial properties. Moreover, it was found that the extract exerted anti-quorum sensing activity and showed QS system inhibition effects on *P.aeruginosa* PAO1 at a moderate level.

Kaempferol was found to be the major constituent of the extract. The computational analysis demonstrated that kaempferol could inhibit the QS system by competitively inhibiting the binding of OdDHL to LasR. However, kaempferol did not exhibit a similar inhibiting affinity towards PqsR by competing with its reference ligand, NHQ. It is important to note that further experimental studies are required to confirm these hypotheses. In future studies, antimicrobial activities of the extract on other microorganisms and in vivo activities need to be investigated.

Author Contributions: BI and EÖ analyzed the antimicrobial activity data and prepared the article. **AR and EA** were involved in the data collection and analysis of the data. **MTM** took part in molecular docking identification and read the outline. **EÖ** was involved in data collection and HPLC analysis of the data. All authors have read and contributed to the article.

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Conflicts of Interest: The authors declare that they have no financial interest.

Studies involving plants: The *R. catharticus L.* plant used in the study was commercially obtained from the Isparta region of Turkey.

Abbreviations

QS: Quorum Sensing; CN: Gentamicin; DMSO: Dimethyl sulfoxide; HPLC: High-Pressure Liquid Chromatography; LB: Luria-Bertani; MHA: Mueller Hinton Agar; OD: Optical Density; PDB: protein data bank; LSD: Least Significant Difference; OdDHL: N-3-Oxo-Dodecanoyl-L-Homoserine Lactone.

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