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The Effect of *Rheum ribes* L. Extracts on Bacterial Communication and Antibacterial Activity

Işkın (*Rheum ribes* L.) ekstraktlarının Bakteriyel İletişim Üzerine Etkisi ve Antibakteriyel Aktivitesi

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Özet

Amaç: Bakteriyel dirençle mücadelede bakteriler arasındaki iletişimin engellenerek virülansın önüne geçilmesi, enfeksiyon hastalıklarıyla mücadelede yeni ve etkili bir seçenek olarak görülmektedir. Birçok bakteri gibi *Pseudomonas aeruginosa* da bu sistemi alkali proteaz, piyosiyanin, fosfolipaz ve endotoksin gibi virülans faktörlerini sentezlenmesinde, ayrıca enfeksiyon hastalıklarında önemli rol oynayan biyofilm oluşumu ve kayma hareketini yaparken kullanmaktadır. **Materyal-Metot:** Yapılan bu çalışmada ışkın (*Rheum ribes* L.) bitkisinin metanol (MeOH), metanol-kloroform (MeOH-$CHCl_3$) ve su (H_2O) ekstraktlarının kontrolü çevreyi algılama sistemi tarafından sağlanan *P. aeruginosa* PA01'in yapmış olduğu kayma hareketi üzerine inhibisyon etkisine bakılmıştır. Ayrıca Gram-pozitif *Bacillus cereus* ATCC 11778, *Enterecoccus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Metisilin-Resistant Staphylococcus aureus* ATCC 43300 ve Gram-negatif *Chromobacterium violaceum* ATCC 12472, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* PA01 bakterileri üzerinde antibakteriyel etkileri araştırılarak minimum inhibisyon konsantrasyonları (MIK) belirlenmiştir. **Bulgular:** Antibakteriyel aktivite sonuçları, tüm ekstraktların çalışmaya dahil edilen Gram-pozitif bakteriler üzerinde farklı oranlarda etkili olup özellikle metanol ekstraktının diğerlerine oranla yüksek aktiviteye (15,3 mm-19,7 mm) sahip olduğunu göstermiştir. Kayma hareketi (swarming motility) üzerine ise metanol ve su ekstraktının inhibisyon etkisi %80 olarak bulunmuş, kloroform ekstraktı ise benzer bir sonuç ile %74 oranında önemli inhibisyon göstermiştir. **Sonuç:** Işkın bitkisi kök ekstraktlarının özellikle Gram pozitif bakteriler üzerinde antibakteriyel özelliğe sahip olduğu, *P. aeruginosa* PA01'in yaptığı kayma hareketini inhibe ettiği ve enfeksiyon hastalıkları ile mücadelede yeni nesil ilaçlara model olabilecek bir bitki olma potansiyeline sahip olabileceği düşünülmektedir.

Anahtar Kelimeler: *Rheum ribes* L. kayma hareketi, çevreyi algılama, PA01, antibakteriyel

Abstract

Objective: The discovery of quorum sensing systems regulating bacterial virulence has afforded a novel opportunity to control infections without interfering with growth. As many bacteria *Pseudomonas aeruginosa* also used this system synthesized of some virulence factor such as alkaline protease, pyocyanin, phospholipase and exotoxin A, biofilm formation and swarming motility. Swarming motility is one of the major virulence factors; it is known to play a role in early biofilm development. **Material-Method:** In this study, inhibitory effect of methanol (MeOH), methanol-chloroform (MeOH-$CHCl_3$) and water (H_2O) extracts of the *Rheum ribes* L. on swarming motility in *P. aeruginosa* PA01 was investigated. And also antibacterial activity and minimum inhibitory concentration (MIC) values were investigated on Gram-positive *Bacillus cereus* ATCC 11778, *Enterecoccus faecalis* ATCC 29212, *Listeria monocytogenes* ATCC 7644, *Staphylococcus aureus* ATCC 25923, *Metisilin-Resistant Staphylococcus aureus* ATCC 43300 and Gram-negative *Chromobacterium violaceum* ATCC 12472, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Pseudomonas aeruginosa* PA01. **Results:** As a result, all extracts showed antibacterial effect on Gram-positive bacteria in different rate and according to swarming motility experiments, the highest inhibitory effect was seen in methanol and water extracts of *Rheum ribes* with 81%. Also chloroform extract showed similar inhibition rate (78%) on swarming motility. **Conclusion:** It is thought that the root extract of the plant has antibacterial properties especially on Gram positive bacteria, inhibits the swarming motility in *P. aeruginosa* PA01 and may have the potential to be a model for new generation drugs in the fight against infectious diseases.

Keywords: *Rheum ribes* L. swarming motility, quorum sensing, PA01, antibacterial

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Introduction

Antibiotics, considered as of the most important inventions of human history, have lost their effects due to the resistance developed as a result of their inappropriate and unnecessary use. Especially in hospitals, hospital infections that develop with multidrug-resistant origins increase mortality rates and cause a lot of additional costs (1). Today, when many antibiotics have become ineffective against bacteria, new strategies to fight bacteria are vital. One of the most emphasized strategies in recent years is the inhibition of communication between bacteria and the other is the use of herbal drugs. Due to the resistance caused by unnecessary and misuse of antibiotics, the insufficiency of many pathogens and the detection of side effects increase the necessity of using plants. The use of plants in treatment is based on ancient history, and as in every part of the world, plants that are considered medically important have been used among the people in our country for centuries. Rheum ribes L., which is in Polygonaceae, is a perennial, herbaceous, wild and grows in Lebanon, Iran, Northern Iraq and some provinces in the eastern part of our country. *R. ribes* L. is the only species of Rheum genus grown in our country. In recent years, *R. ribes* has been among the medicinal plants that have attracted attention and have been frequently researched in pharmacological and microbiological fields (2, 3).

The term "Quorum Sensing-QS" refers to the ability of a microorganism to detect and react to microbial population density, usually the ability to react to production and then to diffusible signal molecules (4). With this system used by Gram-positive and Gram-negative bacteria, various physiological activities such as production of virulence factors, conjugation, antibiotic production, motility, sporulation and biofilm formation are regulated. The QS system signal molecules consist of acyl homoserine lactone (AHL) in Gram-negative bacteria, small peptides in Gram-positive bacteria, and several groups called "autoinducer-2" (AI-2), which can be found in both groups (5, 6). These synthesized molecules are secreted outside the cell and accumulate there and pass through the membrane through passive diffusion, pulse pumps or unique carriers. When enough signals accumulate, the expression of the relevant genes is stimulated and some products appear (7, 8). The system is responsible for the production of virulence factors, even in the form of different systems in Gram negative and Gram positive bacteria (9). For this reason, one of the alternative options against bacteria in recent years is the suppression of the QS system, which is responsible for the production of virulence factors that cause disease in the host via bacteria (7).

P. aeruginosa is one of the bacteria that use this system, and it produces many virulence factors through the system, especially in patients with cystic fibrosis, burn wound infections after burns, neutropenic patients and bacterial patients who have immunocompromised HIV infections, diabetic patients, and those who use intravenous drugs. The multiple resistances observed in *P. aeruginosa* and many bacterial species for many years has made it necessary to develop new treatment strategies and inhibition of the QS system has become the focus of microbiological studies as an option that can serve this purpose. In addition, the use of herbal drugs for this purpose is another strategy in which more intensive studies are still carried out (10).

With this study, the antibacterial effect of methanol, methanol-chloroform and water extracts of R.ribes L. on Gram-positive and Gram-negative bacteria were investigated, and also the inhibition effect on swarming motility responsible for virulence in *P. aeruginosa* was examined.

Methods

Plant material and extraction

Dried root of *R. ribes* L. plant was obtained from Van and the plant sample was powdered (Waring 8011 EB, USA) and 5 g sample was extracted with 50 mL methanol, methanol-chloroform and water solvents. After waiting for 30 minutes in an ultrasonic bath, samples were evaporated under vacuum using a rotary evaporator (Heidolph Hei-Vap Rotary Evaporator, Germany) at 40 ° C.
The residues were weighted and calculated yield (yield (%) = R/S × 100; R: weight of extracted plants residues and S: weight of plant raw sample) (11) and dissolved with DMSO (dimethyl sulfoxide) to determine the amount of product. Extracts were stored at 4 °C for further use.

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Microorganisms

To investigate the antibacterial effect of *R. ribes* L., 5 Gram-positive (*B. cereus* ATCC 11778, *E. faecalis* ATCC 29212, *L. monocytogenes* ATCC 7644, *S. aureus* ATCC 25923, MRSA ATCC 43300) and 4 Gram-negative (*C. violaceum* ATCC 12472, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *P. aeruginosa* PA01) strains were used and *P. aeruginosa* PA01 were included for the anti-quorum sensing effect.

Antibacterial activity test

In the study, agar well method was used considering the "European Committee on Antimicrobial Susceptibility Testing (EUCAST)" criteria to determine the antibacterial activities of *R. ribes* L. extracts (12).

After inoculating 1 ml of bacterial suspension adjusted to 0.5 McFarland (108/ml) (BioSan, Germany) in Müller-Hinton agar medium. Sterile 100 µl of plant extracts were added to the 6 mm diameter wells. They were incubated at 30/37ºC'de for 24 h. Antibacterial activity was determined by measuring the zone diameters around the wells. The tests were performed in three replicates.

Determination of minimal inhibitory concentration (MIC)

MIC values of extracts were performed by using microdilution method with 96-well plate. 100 µl extract was added to the wells contained 100 µl MHB medium and serially diluted two-fold. 10 µl of bacterial suspension adjusted to 0.5 McFarland inoculated each well and plates were incubated 30/37ºC'de for 24 h. (Table 1). Following incubation, microplates were evaluated. The smallest concentration without growth was determined as MIC value.

Table 1. Antibacterial activity of the tested plant extracts (Diameter of inhibition zone-mm)

	***S. aureus* 25923**	***MRSA* 43300**	***B. cereus* 11778**
Gentamicin	14,3c**	14,3b**	13,3c**
MeOH	19,0a	19,7a	19,7a
MeOH -$CHCl_3$	16,0b	14,3b	16,3b
H_2O	14,3c	12,7c	16,3b
	***E. faecalis* 29212**	***L. monocytogenes* 7644**	***C. violaceum*12472**
Gentamicin	15,7b**	18,0a**	16,7a**
MeOH	17,7a	15,3b	14,7b
MeOH -$CHCl_3$	15,3b	11,3c	12,3c
H_2O	13,7c	10,3c	13,0c
	***E. coli* 25922**	***P. aeruginosa* 27853**	***P. aeruginosa* PA01**
Gentamicin	-	-	-
MeOH	-	-	-
MeOH -$CHCl_3$	-	-	-
H_2O	-	-	-

** The difference between the means shown in different letters in the bacteria is significant (*p<0,01*).

Swarming Motility Assay

The medium used for the swarming motility consist of 8 g nutrient broth 1-1, 5 g bacto agar l-1 and 0.5% glucose and different concentration of plant extracts (methanol: 25µg; methanol-chloroform: 9.9 µg; water: 15µg). 2 µl of overnight bacterial culture (PA01) was added center of the medium. After holding the plates to dry at room temperature, they were incubated at 37 ◦ C for 24 h. The swarming motility was assessed by the distance of swarming from the central inoculation site (13).

Statistical analysis

In this study, one way ANOVA was used in three repetitions with JUMP statistical software and the differences between the data were evaluated with LSD "Multiple comparison tests".

Results

Agar well diffusion and MIC values

Different percentage yield was obtained from extracts and according to calculations, percentage yield of methanol, methanol-chloroform and water extracts were 4%, 1.98% and 1.32% respectively. The agar well method was used to investigate the antibacterial effects and Inhibition zone diameters and results obtained are given in Table 1.

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According to the antibacterial activity results, it was observed that all extracts had antibacterial effects at different rates (15.3 mm to 19.7 mm) on the tested Gram positive bacteria. Among the extracts, methanol extract was found to have the highest effect. Among Gram negative bacteria, only the effect of extracts on *C. violaceum* was determined. As with other bacteria, methanol extract has a high antibacterial effect on *C. violaceum*. Antibacterial effects were not observed on other Gram-negative bacteria, *E. coli* and *P. aeruginosa* in tested concentration. The MICs values of extracts on the strains were given in Table 2. The lowest MIC value was detected with the chloroform-methanol extract against *E. faecalis* (0, 06 mg/ml). Other extracts have also close MIC values against *E. faecalis*.

Table2. MIC values of extracts (mg/ml)

Strains/extracts	**$CHCL_3$-MeOH**	**MeOH**	**H_2O**
S. aureus 25923	0,75	1,25	0,75
MRSA 43300	0,75	1,25	0,75
B.cereus 11778	0,5	1,25	0,75
L.monocytogenes 7644	0,12	0,31	0,19
E. faecalis 29212	0,06	0,16	0,09
C. violaceum 12472	0,248	0,625	0,375

Virulence Factors Activity Results

According to the test results the inhibition effect of the extracts on the swarming motility was determined by measuring the swarming in petri (Figure 1- 2).

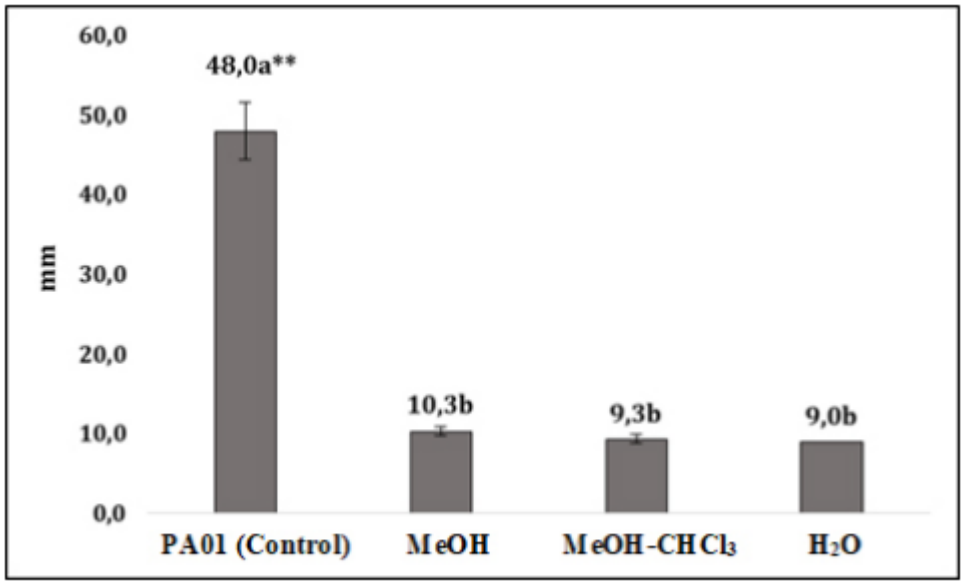

Figure 1. Effect of extracts on swarming motility; ** The difference between the means indicated by different letters is significant. (p<0,01)

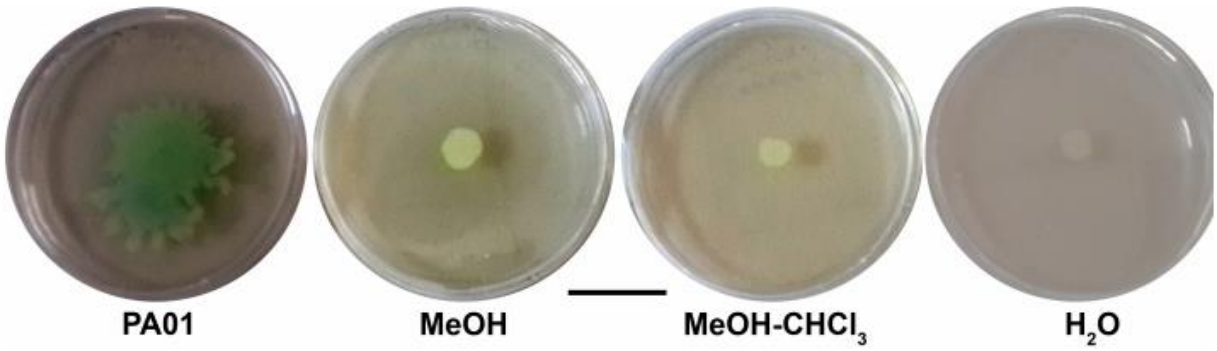

Figure 2. Inhibitory effect of extracts on swarming motility in PA01 (Scala: 30 mm)

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According to these results, it was found that all extracts significantly inhibit the swarming motility of PA01 and the results are statistically significant. The best inhibition was achieved by methanol and water extracts at the rate of 81% while methanol-chloroform extract had a significant inhibition effect with 78% rate.

Disscussion

P. aeruginosa, a multidrug-resistant (MDR) and opportunistic pathogen, is an important cause of hospital infections. The release of virulence factors, which have an important role in the occurrence of *P. aeruginosa*-induced infectious diseases, is carried out by the system known as QS and used by many bacteria (14, 15).

In this study, the inhibition effect of methanol, methanol-chloroform and water root extracts of *R. ribes* L. in swarming motility on *P. aeruginosa* PA01 was investigated and also the antibacterial effects on some Gram-positive and Gram-negative bacteria.

Many antibiotic-resistant bacteria are known to use swarming motility, especially in acute and chronic *P. aeruginosa* infections, it is known to invade tissues by swarming using flagella and pili (16-18).

Although there are many studies with plant on inhibition of swarming, but may be this is the first report inhibition effect on *R. ribes* L. on swarming motility in PA01. The data we obtained with this study showed that different solvent extracts of *R. ribes* L. have a significant inhibition effect on the swarming motility.

According to the antibacterial activity results, it has been determined that the extracts prepared with methanol, chloroform-methanol and water solvents show antibacterial activity against the test microorganisms at different rates and the most sensitive microorganisms among the test microorganisms are Gram-positive *B. cereus* and *S. aureus*. Of the Gram-negative bacteria included in the study, it was observed that all extracts had a similar rate of antibacterial effect only on *C. violaceum*. In many studies with plant essential oils or extracts, it is known that antibacterial effect against Gram-positive bacteria is higher than Gram-negative, while this is known to be related to the complex cell wall structure of Gram-negative bacteria (19, 20).

In a study by Alan et al. (21), they investigated the antibacterial effect of chloroform, hexane, acetone, ethanol and methanol extracts prepared with different parts of the *R. ribes* plant. While the leaf extract of the plant did not affect the test microorganisms, they determined that the methanol and ethanol extracts obtained from the root, stem and seed showed antibacterial activity against the test microorganisms in various proportions, and the most sensitive microorganisms among the test microorganisms were *B. subtilis* ATCC 6633 and *E. aerogenes* ATCC 13048.

Ceylan et al. (22) showed that *R. ribes* L. has antimicrobial properties and the strongest effect is on *Candida albicans* ATCC 90028, while the antifungal effect is higher than the antibacterial effect compared to other microorganisms included in the study. In another study, the antimicrobial effect of fresh root, stem and leaf methanol extracts of *R. ribes* L. plant on some Gram-negative pathogens was investigated, and they found that antibacterial effect on *E. coli*, *P. aeruginosa* and other tested Gram-negative bacteria (23) different from our results. In a similar study consistent with our results, it was found that root extracts prepared with ethanol and water solvents had an antibacterial effect on S. aureus, while there was no antibacterial effect even in high concentrations in Gram-negative *E. coli* and *P. aeruginosa* (24). Literature review and findings obtained as a result of our study showed that extracts prepared with different tissue samples of *R. ribes* L. have an antibacterial effect on bacteria. It is known that the bactericidal effects of plants on microorganisms are caused by components such as secondary metabolites (terpenes, phenolic compounds, nitrogenous compounds) glycosides, alkaloids or essential oils (25, 26). Some studies have shown that *R. ribes* L. have number of phenolic compounds (27, 28).

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Conclusion

Eventually, *R. ribes* L. extracts prepared with different solvents were observed in different rates of antibacterial effect on all studied Gram-positive bacteria, while the highest effect was observed in the extract prepared with methanol. Extracts were found to have a significant inhibition effect on swarming motility on PA01. All these results have suggested that the different part of *R. ribes* L. may offer a potential new therapeutic route for the treatment of bacterial infections by reducing or preventing virulence and pathogenicity of pathogenic bacteria.

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