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Determination of Antimicrobial and Quorum Sensing Inhibition Potentials of Different Types of Berries from Rize

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Abstract

Aim of the study: The rapid increase in antibiotic resistance in recent years poses a major threat to public health. Studies indicate that this resistance issue, expressed in alarming numbers, will lead to significant loss of life, particularly in the 2050s. Therefore, various fruits from the Rize province were screened in this study for their antimicrobial and anti-quorum sensing activities.

Area of study: The investigation took place in İkizdere, situated within the northern part of the Black Sea region in Rize, Türkiye.

Material and methods: While antimicrobial activities of the samples were measured by agar well diffusion method, quorum sensing activity was measured with an agar well and spectrophotometer.

Main results: The results of the study show that the *Cornus mas* plant has potential antimicrobial and quorum sensing properties.

Research highlights: It is thought that it will be important to investigate the different extracts and chemical properties of the *Cornus mas* plant.

Keywords: Quorum Sensing, Pyocyanin, *Cornus mas*

Rize Yöresine ait Farklı Meyve Çeşitlerinin Antimikrobiyal ve Quorum Sensing İnhibisyon Potansiyellerinin Belirlenmesi

Öz

Çalışmanın Amacı: Son yıllarda hızla artan antibiyotik direnç, halk sağlığı için ciddi bir tehdit oluşturmaktadır. Yapılan çalışmalar, bu direnç sorununun korkutucu sayılarla ifade edilen şekilde özellikle 2050'li yıllarda büyük oranlarda can kaybına neden olacağını belirtmektedir. Bu nedenle, çalışma kapsamında Rize iline ait çeşitli meyveler antimikrobiyal ve anti-quorum sensing aktiviteleri açısından taranmıştır.

Çalışma alanı: Bu araştırma, Rize, Türkiye'nin Karadeniz Bölgesi'nin kuzey kısmında yer alan İkizdere'de gerçekleştirildi.

Materyal ve yöntem: Örneklerin antimikrobiyal aktiviteleri agar kuyucuk yöntemi ile ölçülürken, quorum sensing aktivite agar kuyucuk ve spektrofotometre ile ölçüldü.

Temel sonuçlar: Çalışma sonuçları özellikle *Cornus mas* bitkisinin potansiyel antimikrobiyal ve quorum sensing özellik taşıdığını göstermektedir.

Araştırma vurguları: Özellikle *Cornus mas* bitkisinin farklı özütlerinin ve kimyasal özelliklerinin araştırılmasının önemli olacağı düşünülmektedir.

Anahtar Kelimeler: Quorum Sensing, Piyosiyanin, *Cornus mas*

Introduction

Numerous botanical species have been employed in the therapeutic management of various ailments for centuries, persisting as integral components of contemporary medical practices. The attribution of plants as a 'gift of nature' underscores their pivotal contribution to medicinal interventions (Farombi, 2003). Fruits serve as natural antimicrobial agents, complementing their inherent nutritional attributes. For instance, blueberries are recognized for their efficacy in addressing gastrointestinal disorders, while *Cornus mas* is acknowledged for its therapeutic application in urinary infections. Furthermore, the vibrant and aromatic

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qualities of certain fruits are associated with heightened bioactive compound content and quorum sensing activity. Notably, black currant seed extracts containing high molecular weight galactans have been documented for their capacity to impede the adhesion of *Helicobacter pylori* to the human gastric mucosa (Lengsfeld et al., 2004). Puupponen-Pimiä et al. (2001) studies, blackberry (*Rubus chamaemorus*), raspberry (*Rubus idaeus*), blackcurrant (*Ribes aureum*), blackberry (*Vaccinium vitis-idaea*), strawberry (*Fragaria virginiana*), blueberry (*Vaccinium spp*.), cranberry (*Cornus mas*) and sand thistle (*Hippophaerhamnoides*) are reporting the antimicrobial activities of naturally occurring phenolic substances (Puupponen-Pimiä et al., 2001). The investigation revealed that the fruit extracts examined in the study predominantly influenced the proliferation of Gram-negative bacteria, exhibiting limited efficacy against Gram-positive bacterial strains. Specifically, blackberry, raspberry, and strawberry extracts demonstrated pronounced inhibitory effects on the development of *Salmonella*. In contrast, sand thistle and blackcurrant exhibited the least activity against Gram-negative bacteria. A parallel inquiry conducted by Wilkinson et al. (2003) examined the antimicrobial properties of fresh fruits, including raspberry, currant, cranberry, and blueberry, as well as their 100% fruit-derived juices against a diverse array of 12 bacterial strains. The results indicate that these fruits may have potential applications in the purification of water from microbial contaminants in suspected water sources and in prolonging the shelf life of food products (Wilkinson et al., 2003). In another study, it was determined that elderberry (*Sambucus nigra*) and blackcurrant (*Ribes aureum*) juices and concentrates inhibited the growth of *Escherichia coli* and *Staphylococcus aureus* (Werlein et al., 2005).

This investigation systematically examined the antimicrobial and anti-quorum sensing properties of methanol, ethyl acetate, ethanol, and hexane solvents extracted from the fruits of several plant species. These species include *Arbutus unedo* (Big berry), *Aronia melanocarpa* (Russian blueberry), *Cornus mas* (Cranberry), *Fragaria vesca* L. (Wild village strawberry), *Frangula alnus* (Gunpowder tree), *Fructus cynosbati* / *Rosa canina* (Rosehip), *Solanum nigrum* (Rainberry), *Sorbus torminalis* (Maple tree), *Vaccinium myrtillus* (Likapa), and *Vitis labrusca* L. (Izabella scented grape).
The findings of this study could lay the groundwork for more extensive research into the traditional medicinal plant resources of the Rize region. Such studies could explore broader applications in the food and pharmaceutical industries.

Materials and Methods

Collection of Fruits and Preparation of Extracts

The fruits employed in this investigation underwent collection and subsequent storage at a temperature of -20°C following a thorough cleaning process. Extraction of methanol, ethyl acetate, ethanol, and hexane derivatives were achieved through the maceration method (Solanki and Nagori 2012). Concisely, 10 to 20 grams of fruit specimens, stored at -20°C, were weighed, pulverized into a powder using a mortar, and subsequently transferred into flasks. Tenfold volumes of solvents were added to the flasks containing the powdered fruit, and the resultant mixture was subjected to magnetic stirring at room temperature for 48 hours. Following this period, the extract underwent filtration through filter paper and was subsequently evaporated at 40°C in an evaporator.

The resulting extracts were dissolved in Dimethyl sulfoxide (DMSO) within a concentration range of 50 to 100 mg/mL. The same procedural steps were iteratively applied to the remaining solvents. All extracts were preserved at -20°C until their utilization. Given the use of total extracts in the study, the experimental procedures were initiated with an initial extract concentration of 150 mg/mL, progressively decreasing to concentrations of 25, 15, and 10 mg/mL (Table 1).

Table 1. Microorganisms and plants used in the study.

Microorganisms (Antimicrobial Activity)	Microorganisms (Quorum Sensing Inhibition Tests)	Plants
Staphylococcus aureus ATCC 25923	*Chromobacterium violaceum* ATCC 12472	*Arbutus unedo*
Escherichia coli ATCC 25922	*Pseudomonas aeroginosa* PAO1	*Aronia melanocarpa*
Pseudomonas aeroginosa ATCC 27853		*Cornus mas*
Bacillus subtilis ATCC 6633		*Fragaria vesca L.*
Enterococcus faecalis ATCC 29212		*Frangula alnus*
Enterobacter aerogenes ATCC 13048		*Fructus cynosbati / Rosa canina*
Acinetobacter haemolyticus ATCC 19002		*Solanum nigrum*
Klebsiella pneumonia ATCC 13883		*Sorbus torminalis*
Salmonella typhimurium ATCC 14028		*Vaccinium myrtillus*
Candida parapsilosis ATCC 22019		*Vitis Labrusca L.*
Candida albicans ATCC		
Mycobacterium smegmatis ATCC 607		
Chromobacterium violaceum ATCC 12472		

Antibacterial Activity

The antimicrobial efficacy of the substances was evaluated by employing the agar well diffusion method. Cultures of each microbial strain were freshly prepared and adjusted to 0.5 McFarland standards (corresponding to 1×10^8 cfu/mL for bacteria and 1×10^6 cfu/mL for Candida strains), subsequently spread onto the respective media. Fifty microliters of each substance were carefully dispensed into wells created in the agar using a sterile cork borer. Positive controls comprised ampicillin (10 µg/well; for Gram-positive bacteria), gentamicin (10 µg/well; for Gram-negative bacteria), ciprofloxacin (10 µg/well; *M. smegmatis*), and amphotericin B (20 µg/well; *Candida* species). Equivalently, negative controls involved the addition of DMSO at concentrations equivalent to the extracts into the corresponding wells. Incubation of the plates ensued for 18 hours for bacterial strains and 48 hours for Candida strains, following which the plates were scrutinized for the presence of growth inhibition zones (Denev et al., 2014; Woods, 2011; Brown-Elliott et al., 2019; Matuschek et al., 2023).

Anti-quorum Sensing Activity

The sub-minimal inhibitory concentration (Sub-MIC) values of the extracts were determined against the *C. violaceum* ATCC 12472 strain to assess pigment inhibition. Initially, *C. violaceum* was inoculated into 5 mL of Luria-Bertani (LB) medium and incubated for 24 hours at 37°C with agitation at 175 RPM. Following incubation, the cultures were spread onto LB agar plates, dried, and then 50 µL of the predetermined Sub-MIC concentrations of each extract were added to wells created on the agar surface. The presence of anti-quorum sensing properties was determined by observing zones where bacterial growth occurred, but the characteristic purple pigment formation was suppressed.

Pyocyanin Inhibition Activity

Pseudomonas aeruginosa PAO1 was cultivated in 5 mL of Luria-Bertani (LB) broth under conditions of 37°C and 220 RPM for approximately 9 hours. Subsequently, the culture was subjected to a 1:100 dilution in LB broth and transferred to 4 mL culture tubes. Following the addition of the extracts to the culture tubes, the medium volume was adjusted to 5 mL and incubated for 24 hours at 37°C with continuous agitation at 220 RPM. For pigment quantification, 1.5 mL of the culture was extracted and subjected to centrifugation at 13,000 rpm for 5 minutes to precipitate over time. The resulting supernatant was transferred into a clean tube, followed by the addition of 0.9 mL of chloroform. The extraction of pyocyanin pigment was accomplished by vortexing at the highest speed for 30 seconds, followed by a 5-minute incubation at room temperature. The chloroform layer was transferred to a new tube, and 0.3 mL of 0.2 N hydrochloric acid (HCl) was added. After vortexing, the mixture was centrifuged at 13,000 RPM for 5 minutes, and the optical density was measured at 520 nm, focusing on the green top layer. Graphical representations were then generated based on the collected data.

Results and Discussion

Microorganisms engage in intercellular communication by secreting diverse classes of signaling molecules. The accrual of these signaling molecules beyond a certain threshold facilitates the regulation of numerous genes among microorganisms (Abisado et al., 2018). Anti-microbial anti-quorum sensing effects of extracts obtained from some fruits grown widely in and around Rize were investigated on various bacteria and fungi.

Anti-microbial Activity Results of Extracts

The results of the study show that the fruits of the *C. mas* plant have higher activity than the others. In the second place, it is seen that the fruit of *A. unedo* has antimicrobial activity. While *V. myrtillus, F. alnus* were found to have low activity, no antimicrobial activity was found in other fruits (Table 2).

Table 2. Antimicrobial activity results of fruits

	Solvent	*S. aureus*	*B. subtilis*	*E. faecalis*	*E. coli*	*P. aeruginosa*	*A. haemolyticus*	*K. pneumoniae*	*E. aerogenes*	*S. thymurium*	*C. violaceum*	*C. albicans*	*C. parapsilosis*	*M. smegmatis*
C. mas	MeOH	20.0 ±0	14.0 ±1.73	12.0 ±0	10.0 ±2.0	8.33 ±2.51	19.0 ±1.0	10.66 ±3.05		8.33 ±2.30		17.66 ±2.08	-	-
	EtOAc	15.0±0	-	-	10.66 ±2.08	-	15.0 ±0	-	-	-	8.0 ±2.64	12.33 ±0.57	-	-
F. alnus	MeOH	-	-	-	-	-	-	12.0 ±1.73	-	-	-	16.33 ±1.52	-	-
	EtOAc	10.0 ±1.0	9.0 ±1.73	-	-	-	-	-	-	-	-	-	-	-
A. unedo	EtOAc	14.66 ±2.08	12.33 ±1.52	9.0 ±1.73	-	-	21.0 ±0.0	-	-	-	14.0 ±0.0	9.66 ±2.08	-	-
V. myrtillus	EtOAc	9.0 ±1.0	-	-	-	-	-	-	-	-	8.0 ±1.0	-	-	-
Controls		30.33 ±0.47	22.33 ±0.47	15.0 ±0	25.0 ±0	13.0 ±0.47	23.0 ±0	22.0 ±0	19.0 ±0.47	17.0 ±0	30.33 ±0.47	28.0 ±0	25.0 ±0	24.33 ±0.47

The experiments were conducted in triplicate, and the average values of the zone diameters were calculated and presented. Dimethyl sulfoxide (DMSO) served as the negative control, while ampicillin, gentamicin, amphotericin B, and ciprofloxacin were used as positive controls. A zone diameter of 6 mm corresponds to the negative control in the table.

Violasin Inhibition Results

Results of the study showed violasin suppression activity only in *C. mas* and *A. unedo* plants (Table 3.)

Table 3. Violasin suppression activity of plants.

	Solvent	*C.violaceum* 12472
A.unedo	MeOH	+
	EtOAc	+
C. mas	MeOH	+
	EtOAc	+

Pyocyanin Suppression Results

Although the results of pyocyanin suppression from the tested extracts did not yield significant results after three repetitions, some degree of pyocyanin suppression was observed in the methanol extracts of *Fragaria vesca* and *Cornus mas* plants. (Figure 1, 2, 3, 4 and 5).

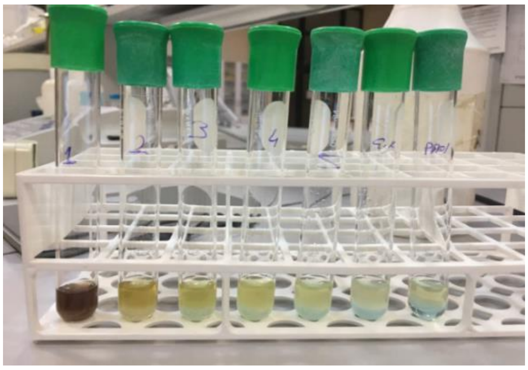

Figure 1. Pyocyanin suppression image of *F. alnus* MeOH extract.

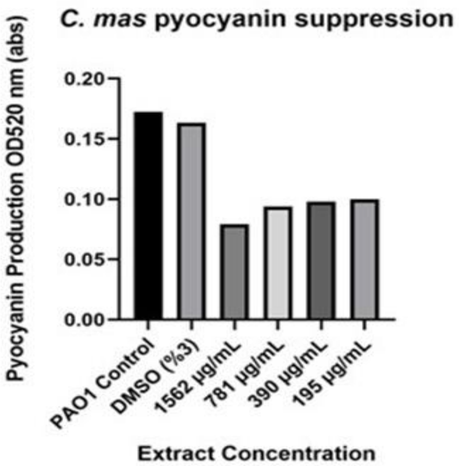

Figure 2. *C. mas* MeOH pyocyanin suppression spectrophotometric results

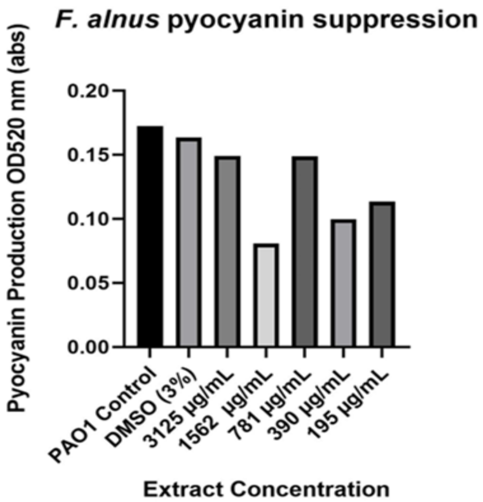

Figure 3. *F. alnus* MeOH pyocyanin suppression spectrophotometric results

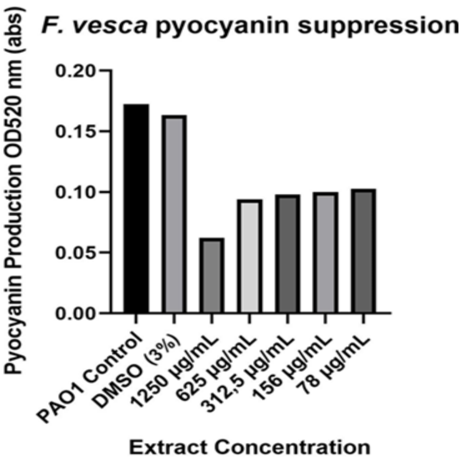

Figure 4. *F. vesca* MeOH pyocyanin suppression spectrophotometric results

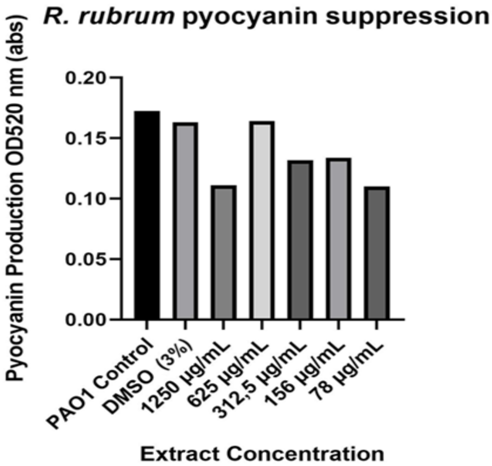

Figure 5. *R. rubrum* MeOH pyocyanin suppression spectrophotometric results

Production of pyocyanin, a virulence factor, from QS steps is a characteristic feature in *P. aeruginosa* PAO1 strain. The study found that the suppression of pyocyanin by the plant tested did not exhibit statistical significance across repeated experiments. Today, with increasing antibiotic resistance, studies focus on the discovery of more effective molecules. At the same time, new strategies are sought in the fight against antibiotics. Quorum sensing is known as signal communication, and current studies are investigating substances that interrupt signal communication in the fight against antimicrobial resistance, thus showing anti-quorum sensing activity.

In light of recent discoveries and numerous past observations, it is anticipated that traditional medicinal plants will persist as a valuable reservoir of alternative antimicrobial agents, addressing the challenges posed by the rise of drug-resistant microbes. Several reports have substantiated the widespread occurrence of drug-resistant bacterial strains in both clinical and food samples (Anbessa et al., 2012; Dugassa et al., 2014 Mulat et al., 2015). The rising prevalence of drug-resistant bacteria, along with the limited availability and high costs of new-generation drugs, has led to an increase in infection-related morbidity and mortality, especially in developing countries such as Ethiopia (Chen et al., 2004). Consequently, the identification of plants exhibiting potent activity against pathogenic microorganisms in the current study, consistent with earlier findings from Ethiopia (Geyid et al., 2005; Taye et al., 2011), reinforces the potential for exploring alternative strategies to manage drug-resistant microbes. As highlighted by various scholars, utilizing plant extracts for disease control not only provides effective outcomes but also offers additional advantages such as reduced production costs, minimal environmental impact, and improved accessibility for rural communities (Savoia, 2012).

Conclusions

The results of the study show that fruits should be screened in terms of different characteristics. It will be screened for different activities, including chemical studies, especially for *C. mas,* which appears to have potential. In addition, it is thought that the chemical profile of the fruits should be determined.

Furthermore, the observed anti-quorum sensing activities in traditional medicinal plants suggest a promising potential for utilizing plant extracts to modulate microbial physiology in a manner beneficial to human health. As a result, Quorum Quenching emerges as a viable alternative strategy to combat bacterial infections, thereby reducing the emergence of multidrug-resistant pathogens. Additionally, considering that plants, like humans and other animals, are frequently exposed to bacterial infections, it is reasonable to assume that plants have evolved sophisticated chemical mechanisms to inhibit biofilm formation and other microbial pathogenic processes.

This investigation represents the first evaluation of the potential use of plants from the Rize region to disrupt microbial cell-cell communication, known as anti-Quorum Sensing activities. However, it is important to highlight that the specific chemical composition of the active compounds and the mechanisms through which these biomolecules interfere with microbial processes were not extensively investigated in this study. Therefore, further research is warranted to elucidate the identity of these active compounds and to understand the detailed mechanisms by which they interact with microbial processes.

Ethics Committee Approval

N/A

Peer-review

Externally peer-reviewed.

Author Contributions

Conceptualization: S.E., A.O.K; Investigation: Ü.Z.Ü.E., İ.D.; Material and Methodology: Ü.Z.Ü.E., İ.D., A.O.K.; Supervision: A.O.K, S.E.; Visualization: Ü.Z.Ü.E., İ.D.; Writing-Original Draft: Ü.Z.Ü.E., İ.D.; Writing-review & Editing: Ü.Z.Ü.E., İ.D.; Other: All authors have read and agreed to the published version of manuscript.

Conflict of Interest

The author has no conflicts of interest to declare.

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