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Antimicrobial and Anti-quorum Sensing Activity of *Geranium sp*.

Geranium sp'nin Antimikrobiyal ve Anti-quorum sensing aktivitesi

Ülkü Zeynep Üreyen Esertaş[1]*, Saliha Ekşi [2]

ABSTRACT

Aim: Antibiotic resistance, which has increased rapidly in recent years, is one of the leading public health threats. Alternative methods are being investigated for effective antibiotics in the fight against resistance. *Geranium* species are widely used for constipation, digestive disorders, and diabetes. It is also known that the plant is used in various public health applications. In line with this information, it was aimed to investigate the antimicrobial and anti-quorum sensing properties of the *Geranium* sp.

Material and Methods: In this study it was aimed to prepare methanol, ethyl acetate, ethanol, and hexane extracts of *Geranium* sp. flower parts belonging to Rize province and investigate their antimicrobial activities by agar well diffusion method against various Gram-negative and Gram-positive bacteria and two fungal species. Anti-quorum sensing activity was determined using *Chromobacterium violaceum* strain.

Results: Methanol extract of *Geranium* sp. showed antimicrobial activity against seven species whereas extraction with ethyl acetate showed antimicrobial activity against eight species. The ethanol extracted samples have the lowest antimicrobial activity so that only six species were affected. Additionally, methanol extract of *Geranium* sp. had violacein inhibition activity.

Conclusions: As a result, it was determined that the plant, especially its methanol extract, has antimicrobial and anti-quorum sensing properties.

Keywords: Violacein, Antimicrobial activity, Quorum sensing, *Geranium* sp.,

ÖZ

Amaç: Son yıllarda hızla artan antibiyotik direnci, halk sağlığı tehditlerinin başında gelmektedir. Dirençle mücadelede etkili antibiyotikler için alternatif yöntemler araştırılmaktadır. Sardunya türleri kabızlık, sindirim bozuklukları ve diyabet için yaygın olarak kullanılmaktadır. Aynı zamanda bitkinin çeşitli halk sağlığı uygulamalarında kullanıldığı bilinmektedir. Bu bilgiler doğrultusunda *Geranium* sp bitkisinin antimikrobiyal ve anti-qorum sensing özelliklerinin araştırılması amaçlanmıştır.

Gereç ve yöntemler: Bu çalışmada, Rize iline ait *Geranium* sp. çiçek kısımlarının metanol, etil asetat, etanol ve hekzan özütlerinin hazırlanması ve agar kuyu difüzyon yöntemi ile çeşitli Gram-negatif ve Gram-pozitif bakterilere ve iki mantar türüne karşı antimikrobiyal aktivitelerinin araştırılması amaçlanmıştır. *Chromobacterium violaceum* suşu kullanılarak anti-quorum algılama aktivitesi belirlenmiştir.

Bulgular: *Geranium* sp.'nin metanol özütü yedi türe karşı antimikrobiyal aktivite gösterirken, etil asetat ile ekstraksiyon sekiz türe karşı antimikrobiyal aktivite göstermiştir. Etanol ile ekstrakte edilen örnekler en düşük antimikrobiyal aktiviteye sahip olduğundan sadece altı tür etkilenmiştir. Ayrıca, *Geranium* sp.'nin metanol özütü violacein inhibisyon aktivitesine sahiptir.

Sonuç: Sonuç olarak, bitkinin özellikle metanol öztünün antimikrobiyal ve anti-quorum algılama özelliklerine sahip olduğu belirlendi.

Anahtar kelimeler: Viyolasin, Antimikrobiyal aktivite, Quorum algılama, *Geranium* sp.,

1.Department of Medical Microbiology Ağrı Ibrahim Çeçen University
2.Medical Microbiology Recep Tayyip Erdoğan University

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**Corresponder author*
Ülkü Zeynep Üreyen Esertaş
Department of Medical Microbiology Ağrı Ibrahim Çeçen University, Ağrı TURKEY
E-mail: uzesertas@agri.edu.tr
ORCID ID: 0000-0001-9897-5313

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Introduction

Antibiotics are critical agents in the treatment of bacterial infections, revolutionizing modern medicine since their discovery. They function by inhibiting bacterial growth or killing bacteria, thus playing a vital role in managing infectious diseases and preventing complications from surgeries and other medical procedures. However, the emergence of antibiotic resistance (ABR) poses a significant global health threat, as bacteria evolve mechanisms to withstand the effects of these drugs. Factors contributing to this resistance include overuse and misuse of antibiotics, inadequate patient education, and the lack of new antibiotic development to keep pace with resistant strains (1-3). The World Health Organization has identified antibiotic resistance as one of the top ten global health threats, emphasizing the urgent need for effective strategies to combat this issue (3).

The properties of antibiotics derived from plants have garnered increasing interest as potential alternatives to traditional antibiotics. Plants have evolved complex chemical defenses that allow them to combat pathogens, which can be harnessed for medicinal purposes. Research has shown that various plant extracts possess antimicrobial properties, with some exhibiting synergistic effects when combined with conventional antibiotics (4, 5). These plant-derived compounds can disrupt bacterial cell membranes, inhibit essential metabolic pathways, and even enhance the efficacy of existing antibiotics (5). For instance, studies have highlighted the potential of specific plants, such as *Aloe vera*, in producing antibacterial agents that could serve as adjuncts in treating resistant infections (6). The exploration of these natural products not only offers a promising avenue for developing new therapeutic agents but also aligns with the growing trend towards sustainable and holistic approaches in medicine.

Thus far, while antibiotics remain indispensable in modern healthcare, the rise of antibiotic resistance necessitates a multifaceted approach that includes prudent antibiotic use and the exploration of alternative sources, such as plant-derived compounds. This dual strategy may help mitigate the impact of resistance and ensure the continued effectiveness of antibiotics in treating bacterial infections. Developing new and effective antimicrobials is of great importance in combating antibiotic resistance. Simultaneously, new strategies for fighting resistance are being researched. Among these strategies, quorum sensing, a bacterial communication system, plays a significant role. Plants have historically been prominent in active substance research (7, 8). The genus *Geranium*, commonly known as crane's bill, includes several species that are referred to by various local names such as needle herb, bramble, shepherd's herb, minute herb, stork's foot, stork's beak, stork's bill, clock herb, and hour hand herb. In the Aegean region, some species' leaves are used as vegetables. *Geranium* species are traditionally used for their hemostatic, laxative, diuretic, tonic, digestive disorder, and diabetes management properties (9). *Geranium mexicanum* is reported to be preferred in Mexican traditional medicine for the treatment of tonsillitis, cough, whooping cough, urticaria, dysentery, and diarrhea (10). Extracts prepared from the roots and aerial parts of *G. mexicanum*, used for ailments such as stomach pain, diarrhea, and dysentery, have been noted to exhibit activity against diarrhea and dysentery pathogens. Another study reports that *Geranium dielsianum*, used in alternative medicine in South America for treating diabetes, lowers blood glucose levels (11). In Eastern medicine, *Geranium thunbergii* is commonly used not only for its hemostatic and anti-diarrheal properties but also for sterilization purposes (12).

In this context, the antimicrobial and anti-quorum sensing activities of extracts prepared from *Geranium* sp. using four different solvents were investigated within the scope of the study.

Materials and Methods

Preparation of plant extracts

Geranium sp. samples were collected from Rize Ovit Plateau in July 2018. Genus's identification was performed by academicians from Botany department at Recep Tayyip Erdoğan University. Herbarium procedures were carried out with the assistance of Prof. Dr. Vagıf Atamov. The extract was prepared using the maceration method with flower part of the plant (13). Final sample concentration was set up as 10 mg/mL of the plant extraction.

Antimicrobial activity

Antimicrobial activity of *Geranium* sp. extracts was tested against *Staphylococcus aureus* ATCC 25923, *Escherichia coli* American Type Culture Collection (ATCC) 25922 *Pseudomonas aeruginosa* ATCC 27853, *Bacillus subtilis* ATCC 6633, *Bacillus cereus* ATCC 14579, *Enterococcus faecalis* ATCC 29212, *Enterobacter aerogenes* ATCC 13048, *Yersinia pseudotuberculosis* ATCC 6904, *Acinetobacter haemolyticus* ATCC 19002, *Klebsiella pneumoniae* ATCC 13883, *Salmonella typhimurium* ATCC 14028, *Candida parapsilosis* ATCC 22019, *Candida albicans* ATCC 10231, *Mycobacterium smegmatis* ATCC 607, *C. violaceum* ATCC 12472. All strains were ATCC standard microorganisms and were obtained from the culture collection of the Department of Medical Microbiology, Medicine Faculty, Black Sea Technical University Trabzon/TURKEY.

Antimicrobial activity of *Geranium* sp. extracts was evaluated using the agar well diffusion method. The agar well diffusion method involves preparing agar plates and inoculating them with a microbial suspension. Before the experiments, fresh cultures of bacteria were prepared on Müeller Hinton agar (MHA). During the experiment, bacteria were prepared at a density of 0.5 McFarland *Candida* species and 1 McFarland. Then, bacteria were spread on MHA media with sterile swabs. 50 µL of the extracts at 10 mg/mL stock concentration were placed in the wells and the bacteria were incubated at 37 °C overnight and 2 nights with fungus. Wells were created in the agar, and a test substance is added to each well. After incubating the plates to allow microbial growth, the zones of inhibition around the wells are measured to assess the antimicrobial activity of the substance. The size of these zones indicates how effective the test compound is against microorganisms. Fifty microliters of *Geranium* extracts were added to the wells. Fungi were incubated for two days and bacteria for one day. Activity was determined by measuring the zone diameters around the wells (14, 15). All experiments were performed as three replicates. Ampicillin, gentamicin, ciprofloxacin and amphotericin B (200µg/mL) use for antimicrobial activity test for positive controls. Dimethyl sulfoxide (DMSO) negative control for the experiments.

The extracts were tested within a concentration range of 5 mg/mL to 39.06 µg/mL. For this purpose, 100 µL of the extract from the 10 mg/mL working stock solution was taken and transferred to the first well containing to 100 µL media. Subsequently, a two-fold serial dilution was performed starting from the first well. For bacteria, Mueller Hinton broth-II was utilized, while Brain Heart Infusion (BHI) broth was employed for *M. smegmatis*. *Candida* species were tested in RPMI (Roswell Park Memorial Institute)1640 with 0.2% glucose following the guidelines of EUCAST (2022). MIC values represented the lowest concentration of substances preventing visible growth of test strain. To determine Minimum Bactericidal Concentration (MBC), 50-µL samples were extracted from the MIC well and the preceding three wells. These samples were then plated on media specific to each microorganism and incubated at 37 °C for the respective durations. The MBC was defined as the lowest concentration inhibiting viable cell growth on the plate (16).

Anti-quorum sensing activity

Chromobacterium sp. were then cultured in 5 mL of LB medium, shaken at 175 rpm, and incubated for 24 hours at 37 °C. Subsequently, 50 µL of each culture was inoculated into 5 mL of soft LB agar, with C6-AHL and C7-AHL added for CV026, and 25 µL (1 mM) of C12-AHL added for VIR07 among the indicator strains. These cultures were applied to LB agar plates, allowed to dry, and 6 mm wells were opened, followed by the addition of 50 µL of determined Sub-MIC concentrations of each extract. The presence of anti-quorum sensing activity was identified by assessing zones where bacterial growth continuity was observed, while the formation of the purple pigment was suppressed, as described in previous studies (17, 18). Vanilla used as positive control for the quorum sensing activity experiments respectively. Dimethyl sulfoxide (DMSO) negative control for the experiments.

Statistical analysis

Results were presented as mean values and standard error (mean±SE). Data were tested using GraphPad 9.0 (GraphPad Software, Inc., La Jolla, CA, USA). Statistical analysis of the results was based on chi-square test. Significant differences were statistically considered at the level of $p<0.05$ otherwise given.

Results

Antimicrobial activity results

The antibacterial activity of *Geranium* sp. was found to be particularly high against *S. aureus* and *A. haemolyticus*, while it also exhibited antifungal activity against both *Candida* sp. (Table 1).

Table 1. Antimicrobial activity results of *Geranium* sp.

	Ger			Controls
	MeOH	EtOAc	EtOH	
S. aureus	20.33 ±0.57	20±0.0	20±0.0	30.33±0.47
B. subtilis	13±3.6	12.66±1.52	-	22.33±0.47
E. faecalis	-	15±1.0	10.3±0.57	15±0
E. coli	-	-	-	25±0
P. aeruginosa	-	-	-	13±0.47
A. haemolyticus	16±0.0	20±0.0	20±1.0	23±0
K. pneumoniae	15±1.73	-	-	22±0
E. aerogenes	-	-	-	19±0.47
S. typhimurium	-	-	-	17±0
C. violaceum	24±0.0	13.33±2.30	-	30.33±0.47
C. albicans	22±2.0	18.66±2.08	17±0.0	28±0
C. parapsilosis	17±1.73	16±1.0	14.33±1.15	25±0
M. smegmatis	-	14.33±2.08	9±2.0	24.33±0.47

Data was expressed in mm. -: No activity ±: Standard deviation

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values of *Geranium* sp. were found to be lowest for *C. violaceum* and *C. albicans* (Table 2).

Anti-quorum sensing activity results

Anti-quorum sensing activity was determined and only the extract of *Geranium* sp. prepared in MeOH exhibited violacein suppression activity (Table 3).

Table 2. MIC and MBC results of Geranium sp.

		S.aureus	*A. haemolyticus*	*K. pneumoniae*	*C. violaceum*	*C. albicans*	*C. parapsilosis*
MeOH	MIC	125	250	250	31.2	62.5	250
	MBC	250	500	500	62.5	1285	500
EtOAc	MIC	250	250	-	-	250	500
	MBC	500	500	-	-	500	1000
EtOH	MIC	250	250	-	-	250	-
	MBC	500	500	-	-	500	-

Data was expressed in µg/mL. - : No activity.

Table 3. Anti-quorum sensing activity results

	C. violaceum 12472	*C. violaceum* 31532	*C. violaceum* VIR07	*C. violaceum* CV026
MeOH	+	+	+	+
EtOAc	-	-	-	-
EtOH	-	-	-	-
Hxn	-	-	-	-

+: Showed activity -: No activity was observed

Discussion

Uncontrolled and indiscriminate consumption of antibiotics leads to antibiotic resistance, posing a significant threat to public health. Resistance to beta-lactam and glycopeptide antibiotics, especially in microorganisms such as *S. aureus*, is increasing and is a serious concern. Discovery of new compounds that may be effective in combating resistance is of great importance. Current statistics highlight the need for non-toxic, affordable and accessible therapeutics for treating infectious diseases (19).

Geranium species have received significant attention due to their antimicrobial properties attributed to various bioactive compounds present in their essential oils. In antimicrobial activity studies, compounds designed using *Pelargonium graveolens,* a member of the *Geraniaceae* family, were investigated and reported to be particularly effective on *S. aureus* and *E. coli* (20). Bigos et al. (2012) found that oils obtained from the *Geranium* plant were effective against *S. aureus* (21). Although crude extract of *Geranium* sp. was used in our study, similarly high activity on *S. aureus* was observed. Considering this data, the effect of *Geranium* extracts on *S. aureus* seems promising. At the same time, our study results reveal that *Geranium* MeOH, EtOAc, EtOH extracts show activity against *A. haemolyticus* and *Candida* species.

Like our results, researchers have shown that the antimicrobial activity of *Geranium* essential oil is not uniform across different bacterial strains. For example, studies have reported varying degrees of susceptibility between resistant and susceptible strains of *Enterococcus* spp. (22, 23). The ability of *Geranium* essential oils to inhibit the growth of *C. albicans* has also been documented, highlighting its potential as a natural antifungal agent. The presence of phenolic compounds in *Geranium* extracts has been associated with high antibacterial properties due to the ability of these compounds to disrupt microbial cell membranes and interfere with basic metabolic processes (24).

In addition to antimicrobial activity, *Geranium* species have been found to exhibit different activities, which may contribute to their overall therapeutic potential (25). These multiple activities, antimicrobial, anti-inflammatory, antibiofilm and antioxidant, position *Geranium* as a valuable candidate for a variety of applications, including food preservation and therapeutic formulations aimed at treating infections and inflammatory conditions (26).

The safety profile of *Geranium* essential oil is remarkable as it is generally recognized as safe (GRAS) by regulatory authorities and is suitable for use in food and cosmetic products (25, 26). The industrial importance of *Geranium* extends beyond its antimicrobial properties; it is also used in perfumery and aromatherapy, further highlighting its versatility (27).

Especially in recent years, screening for inhibition of the mechanism called quorum sensing, which involves the communication system of bacteria, has been rapidly increasing in new therapeutic research. Bacteria are thought to become pathogenic through quorum sensing. Therefore, the detection of substances showing anti-quorum sensing activity is of great importance. It is predicted that the *Geraniaceae* family, which has been presented with different biological activities in literature, also has the potential for quorum sensing. At the same time, bioactive compounds found in *Geranium* sp. may also synergize with other natural anti-quorum sensing agents, increasing their overall efficacy against bacterial pathogens. This multifaceted approach not only targets the bacterial communication systems but also reduces oxidative stress, a factor that often contributes to bacterial pathogenicity (28).

Literature review reveals that *Geranium* species have not been extensively investigated for their anti-quorum sensing activities (29). Especially in recent years, much work has been done to increase quorum detection screening (30). Elmanama and Al Reefi (2017) investigated the antimicrobial and anti-quorum sensing activities of *Pelargonium hortorum* and *P. graveolens* from the *Geraniaceae* family and stated that P. hortorum in particular had potential (31). It has been reported that the inhibition of violacein by *Pelargonium sidoides*, which belongs to the *Geraniaceae* family, is ≥80% at ≥ 250 µL (26). Another study with P. hortorum revealed the quorum sensing activity of the plant at the genetic level and demonstrated its antimicrobial activity (32). Literature data show that especially *Pelargonium* species from the *Geraniaceae* family are frequently studied. Our study presents a comprehensive preliminary screening by including 4 different solvents of the species *Geranium* sp. At the same time, the study data showed that *Geranium* sp. MeOH extract suppressed violacein pigment production in *C. violaceum* species, indicating its potential to inhibit bacterial communication systems. In line with these results, it is planned to use MeOH extract for different studies and to advance the study in future research.

Conclusion

The rising threat of antibiotic resistance calls for innovative approaches to combat bacterial infections. *Geranium* species, with their rich bioactive compound content, offer promising alternatives to conventional antibiotics. This study demonstrates the antimicrobial potential of *Geranium* extracts, particularly against *S. aureus*, *A. haemolyticus*, and *Candida* species. Furthermore, the methanol extract of *Geranium* exhibited significant anti-quorum sensing activity, which is crucial in inhibiting bacterial communication systems that lead to pathogenicity.

The findings align with existing research that highlights the efficacy of plant-derived compounds, particularly from the *Geraniaceae* family, in disrupting microbial growth and communication. As natural products gain increasing attention in the search for sustainable and non-toxic therapeutics, *Geranium* species could play a significant role in the development of alternative antimicrobial agents. Further investigation is necessary to fully understand the scope of *Geranium*'s anti-quorum sensing capabilities and to explore potential synergistic effects with other natural compounds. This research provides a foundation for future studies aimed at developing plant-based antimicrobials, which could mitigate the global antibiotic resistance crisis while offering safer and more accessible treatment options.

Conflict of Interest

The authors affirmed no conflicts of interest with respect to the publication and/or authorship of this article.

Author Contributions

SE, ÜZÜE: Investigation; conceptualization; funding acquisition; project administration. **ÜZÜE:** Investigation; conceptualization; methodology; writing-original draft; writing-review & editing.

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Data Availability

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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