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Antibacterial Potential of Betel (*Piper betle*) Essential Oil Against Antibiotic-Resistant *Serratia* spp. Isolates from Ready-to-Eat Seafood Salads

Dilek Kahraman Yılmaz[1]*, Melike Avdan[2]

Abstract: This study aimed to evaluate the antibiotic resistance profiles of *Serratia* species isolated from ready-to-eat seafood salads sold in markets in Türkiye and the antibactrial activity of *Piper betle* leaf essential oil against *Serratia* isolates. All seven different *Serratia* isolates identified at the molecular level were determined to be resistant to the antibiotic cephalothin. In addition, *S. liquefaciens* DKY-SS3 strain was found to be resistant to a total of four antibiotics, three of which were beta-lactam antibiotics (cephalothin, ampicillin, and amoxicillin-clavulanic acid) and one was the folic acid synthesis inhibitor trimethoprim-sulfamethoxazole. The resistance profiles obtained were found to be consistent with the intrinsic resistance mechanisms reported in the literature. In the context of alternative preservative strategies, the *P. betle* leaf essential oil used in the study showed strong antibacterial activity against all *Serratia* strains. Inhibition zone diameters and low values of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) obtained in disk diffusion and microdilution tests confirmed the effectiveness of the oil. In the future, it is recommended that the applicability of this oil to food products, its effect on shelf life, and its reliability be investigated.

Keywords: Seafood, Food Pathogens, Spoilage Microorganisms, Facultative Anaerobes.

Tüketime Hazır Deniz Ürünleri Salatalarından İzole Edilen Antibiyotik Dirençli *Serratia* spp. İzolatlarına Karşı Betel (*Piper betle*) Esansiyel Yağının Antibakteriyel Potansiyeli

Özet: Bu çalışma Türkiye'de marketlerde satışa sunulan; tüketime hazır deniz ürünleri salatalarından izole edilen *Serratia* türlerinin, antibiyotik direnç profillerini ve bu izolatlara karşı *P. betle* yaprağı esansiyel yağının antibakteriyal etkinliğini değerlendirmeyi amaçlamıştır. Moleküler düzeyde tanımlanan yedi farklı *Serratia* izolatının tamamının cephalothin (KF) antibiyotiğine karşı dirençli olduğu belirlenmiştir. Ayrıca, *S. liquefaciens* DKY-SS3 suşunun, üçü beta-laktam grubu antibiyotik (sefalotin, ampisilin ve amoksisilin-klavulanik asit), biri folik asit sentezi inhibitörü trimetoprim-sulfametoksazol olmak üzere toplam dört antibiyotiğe karşı dirençli olduğu tespit edilmiştir. Elde edilen direnç profilleri, literatürde bildirilen intrinsik direnç mekanizmaları ile uyumlu bulunmuştur. Alternatif koruyucu stratejiler bağlamında, çalışmada kullanılan *P. betle* yaprağı esansiyel yağı, tüm *Serratia* suşları üzerinde güçlü antibakteriyal etki göstermiştir. Disk difüzyon ve mikrodilüsyon testlerinde elde edilen inhibisyon zon çapları ile düşük Minimum İnhibitör Konsantrasyon (MIC) ve Minimum Bakterisidal Konsantrasyon (MBC) değerleri, yağın etkinliğini doğrulamıştır. Gelecekte bu yağın gıda ürünlerine uygulanabilirliği, raf ömrü üzerine etkisi ve güvenilirliği gibi yönlerinin araştırılması önerilmektedir.

Anahtar Kelimeler: Deniz Ürünleri, Gıda Patojenleri, Bozulma Mikroorganizmaları, Fakültatif Anaeroblar.

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1 Çanakkale Onsekiz Mart Üniversitesi, Deniz Bilimleri ve Teknolojileri Fakültesi, Deniz Biyolojisi Bölümü, dilek.kahramanyilmaz@comu.edu.tr
2 Çanakkale Onsekiz Mart Üniversitesi, Lisansüstü Eğitim Enstitüsü melkeavdn@gmail.com
***Sorumlu yazar:** Dilek Kahraman Yılmaz dilek.kahramanyilmaz@comu.edu.tr

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1. Introduction

The demand for ready-to-eat foods is constantly increasing. The microbiological safety of food products will always be important. Seafood is especially valuable because it is rich in essential fatty acids. Polyunsaturated oils must be prepared under very meticulous conditions as they can spoil quickly. In case of improper processing or storage conditions, they can also be contaminated with spoilage agent microorganisms.

The genus *Serratia*, a member of the order Enterobacterales, includes Gram-negative, facultatively anaerobic, non-spore-forming, rod-shaped bacteria belonging to the family Yersiniaceae. *Serratia* species are widespread in many ecological niches, including soil, water, insects, fish, rodents, and humans (Grimont & Grimont 1978; Labbate et al., 2004; Shymialevich et al., 2024). This genus can grow at low temperatures and secrete thermostable proteases and lipases, causing the formation of biogenic amines such as cadaverine and putrescine (Oktariani et al., 2022). It is known that *Serratia* members are among the dominant microorganisms in seafood and play a key role in product spoilage (Begrem et al., 2021).

There are studies that show *Serratia* species grow especially under modified atmosphere packaging (MAP) in the presence of high temperatures and CO_2 (Anagnostopoulos et al., 2023; Chen et al., 2024). It has been shown that volatile compounds such as trimethylamine (TMA), methanethiol, 2-butanol, 2,3-butanedione, ethyl acetate, 1-propanol, acetaldehyde, 1-penten-3-ol, 2-methyl-1-butanol, and pentanal are formed due to *Serratia* contamination (Jaffrès et al., 2011). In addition, some species of *Serratia* can form toxic biogenic amines, such as histamine, which are seafood contaminants, through the action of the histidine decarboxylase enzyme. It is reported that this situation poses a serious risk to public health (Oktariani et al., 2022).

It is important to know that *Serratia* microorganisms cause potential food poisoning along with spoilage. In terms of food safety, the resistance potential of microorganisms found in seafood to antibiotics should also be carefully evaluated. In this context, the use of plant-based essential oils as alternative food additives in the fight against antimicrobial resistance is an important research topic. The phenolic compound-rich content of the essential oil obtained from the *P. betle* leaf has shown that it can be effective against various food pathogens and spoilage agents (Nayaka et al., 2021; Tran et al., 2023). Studies on the antimicrobial effects of the *P. betle* plant and its components have been limited only to *Serratia marcescens* strains (Srinivasan et al., 2016; Srinivasan et al., 2018). No study has been found in the literature examining the potential effects of *P. betle* on other *Serratia* species.

The aim of this study was to determine the antibiotic susceptibility profiles of *Serratia* species isolated from ready-to-eat seafood salads sold in the Turkish market and to evaluate the antibacterial effect of *P. betle* leaf essential oil against *Serratia* isolates.

2. Materials and Methods

2.1. Ready-to-eat seafood salad samples

In the study, ready-to-eat seafood salads produced by different companies offered for sale in the market were used. Three different companies were selected, and 10 packages (30 packages in total) of salad were provided by each company. The products were brought to the Microbiology Laboratory of the Faculty of Marine Sciences and Technology under a cold chain in accordance with the storage conditions (0-4℃).

2.2. Bacterial isolation

For bacterial isolation, 10 g of ready-to-eat seafood salad was taken and homogenized in 90 ml of peptone water for 2 minutes. Ten-fold dilutions up to 10^2 were prepared from the prepared homogenate. Violet Red Bile Glucose Agar (VRBG) was used to isolate bacteria belonging to the Enterobacteriaceae family. After the dilution process, spread and pour plate cultivation methods were applied. In the spread plate method, 0.1 ml of the sample was taken and inoculated into VRBG medium with the help of a Drigalski spatula. In the pour plate method, 10 g of the sample was homogenized with 90 mL of peptone water, and 1 mL of the resulting suspension was transferred into a sterile Petri dish. Then, 18 mL of VRBG medium cooled to 45 °C was added. The mediums were incubated at 35 ℃ for 48 hours. Pure bacterial isolates produced in tryptic soy broth were stored in cryogenic tubes in the presence of 30% glycerol at -80℃ until their identification and antibacterial analysis.

2.3. Bacterial identification

Species level identification of bacterial strains used in the study was performed in the same way as the method

applied in our previous studies (Kahraman Yılmaz & Berik, 2024). DNA isolations were performed using a commercial kit (GeneMATRIX Bacterial & Yeast DNA Isolation Kit, EurX, Poland). The concentration and purity of the obtained DNAs were measured spectrophotometrically with the Nanodrop 2000 device (Thermo Scientific, USA).

For species identification, the 16S rRNA gene region was amplified by PCR using the universal 27F–1492R primer pair. PCR products were confirmed by agarose gel electrophoresis, and then sequencing was performed by BM Labosis (Ankara, Türkiye). The obtained sequence data were edited with BioEdit software, and species identifications were made using the BLASTN tool in the GenBank database. All bacterial strains are kept as stock cultures at -80°C in the Ecotoxicology Research Laboratory of Çanakkale Onsekiz Mart University, Faculty of Marine Sciences and Technology.

2.4. Antibiotic susceptibility tests

2.4.1. Disk diffusion test

Kirby-Bauer disk diffusion method was applied in antibiotic susceptibility tests (Bauer et al., 1966). Bacterial isolates growth from stock cultures were multiplied at least twice on tryptic soy agar medium. The best colony morphology in terms of growth were selected and transferred to the Mueller-Hinton agar medium. Colonies that showed good growth in this medium were taken, and the bacterial density was adjusted according to 0.5 McFarland standard in the Mueller-Hinton liquid medium. Then, inoculation was made on Mueller-Hinton agar plates using sterile cotton swabs from this adjusted suspension.

After the final inoculation process on the plates, selected disks representing different antibiotic groups were placed in Petri dishes. The antibiotics used are:

- Penicillins: Ampicillin (10 µg) (AM) and Amoxicillin-clavulanic acid (30 µg) (AMC)
- Cephalosporins: Cefotaxime (30 µg) (CTX), Ceftriaxone (30 µg) (CRO), Cephalothin (30 µg) (KF)
- Aminoglycosides: Gentamicin (10 µg) (CN)
- Carbapenems: Imipenem (10 µg) (IPM), Meropenem (10 µg) (MEM)
- Tetracyclines: Doxycycline (30 µg) (DO)
- Folic acid synthesis pathway inhibitors: Trimethoprim-sulfamethoxazole (1.25/23.75 µg) (SXT)
- Phenocols: Chloramphenicol (30 µg) (C)
- Quinolones and Fluoroquinolones: Ciprofloxacin (5 µg) (CIP), Levofloxacin (5 µg) (LEV), Nalidixic acid (30 µg) (NA)

All antibiotic susceptibility tests were conducted using the standard disk diffusion method, and zone diameters against selected antibiotics, especially for isolates belonging to the Enterobacteriaceae family, were evaluated according to CLSI (Clinical and Laboratory Standards Institute) criteria (CLSI, 2020). The inhibition zone diameters formed at the end of the incubation period were measured and the resistance status of the bacteria against antibiotics was determined. In order to ensure the validity of the tests, *Escherichia coli* ATCC 25922 was used as a control strain.

2.5. Essential oil and chemical composition analysis

The *P. betle* leaf essential oil used in this study was supplied by ORLIFE GLOBAL İÇ VE DIŞ TİCARET A.Ş. (Üsküdar, İstanbul, Türkiye). The chemical composition of the essential oil was analyzed based on the GC-MS (gas chromatography-mass spectrometry) protocol specified in our previously published study (Kahraman Yılmaz & Berik, 2023). During the analyses, Shimadzu GCMS-QP 2010 ULTRA brand device and Rxi-5 ms capillary column were preferred. Helium was used as the carrier gas and the operating parameters of the device were adjusted according to the standard GC-MS conditions commonly applied in essential oils. The obtained chromatograms were compared with the Wiley W9N11 mass spectral library for the identification of the components.

As a result of this analysis, the main chemical components of *P. betle* leaf essential oil were determined and recorded to evaluate their relationship with antibacterial activity. The antibacterial effects of some phenolic and aromatic compounds in the composition of the essential oil on the tested *Serratia* isolates were investigated.

2.6. Antibacterial activity tests

2.6.1. Disk diffusion test

In this study, the antibacterial activity of *P. betle* leaf essential oil was evaluated on seven different *Serratia* strains isolated from seafood salad samples. In the application, the Kirby-Bauer disk diffusion method was used as the basis, and experimental procedures were carried out in accordance with standard procedures (Bauer et al., 1966).

In the application of the test, firstly, 10 µL of *P. betle* leaf essential oil was applied to sterile empty disks (Oxoid, CT0998B). Disks were dried in sterile 96-well plates under aseptic conditions in order to eliminate the risk of contamination. Completely dried disks were carefully placed on Mueller-Hinton agar surfaces, which were previously inoculated with *Serratia* strains whose density was adjusted in Mueller-Hinton broth according to the 0.5 McFarland standard, using sterile forceps.

After the discs were placed, the plates were incubated for 24–36 hours at 35 °C. At the end of the incubation period, the diameters of the inhibition zones formed by the effect of the essential oil were measured in millimeters, and the antibacterial activity was graded. The classification was made according to the zone diameter criteria suggested by Rota et al. (2008): zones ≥20 mm were evaluated as "strong inhibitory," zones 12–19 mm as "moderate inhibitory," and zones <12 mm as "ineffective."

2.6.2. Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Test

Minimum inhibitory concentration (MIC) analysis was performed using *P. betle* essential oil according to the microdilution method reported by the Clinical and Laboratory Standards Institute (CLSI, 2024). The stock solution of the essential oil was prepared at a concentration of 10% and dissolved in a suitable liquid medium containing 6% DMSO and 0.5% Tween 80 (Turgis et al., 2012). This stock solution was mixed in 96-well microplates as 50 µL medium and 50 µL stock solution, and two-fold serial dilution was applied.

A bacterial suspension of approximately 10^5 CFU/mL was also added to each well as 50 µL. Thus, the final concentration of *P. betle* essential oil was reduced from 2.5% to 0.001221%. Three control groups were created in the experiment:

1. Positive control: 100 µL of medium containing bacteria only,
2. Solvent control: 100 µL of the essential oil mixture prepared with DMSO and Tween 80, without bacterial inoculation,
3. Negative control: 100 µL of medium without bacteria.

The plates were incubated at the appropriate incubation temperature for 24 hours. MIC values were recorded as the lowest concentration of essential oil at which no visible bacterial growth occurred.

For the determination of the minimum bactericidal concentration (MBC), samples were taken from the MIC value and the two dilutions at higher concentrations before it and inoculated on solid media and the lowest concentration at which no viable bacteria remained after 24-36 hours of incubation was determined as the MBC value (Schwalbe et al., 2007).

2.3. Statistical Analysis

Inhibition zone diameters (in millimeters) were measured using the disk diffusion method. All tests were performed in triplicate using a single concentration of *Piper betle* essential oil for each bacterium. The results were processed using Microsoft Excel 2016. Results are expressed as the mean ± standard deviation (SD) of three independent measurements. For MIC and MBC values, these values are reported without indicators of variability since the same results were obtained between replicates.

3. Results

3.1. Bacterial identifications

Sixty-three bacterial isolates were isolated, and 12 of which were determined to belong to the Enterobacteriaceae family by conventional methods. Among the Enterobacteriaceae isolates, seven were further characterized and confirmed to belong to the *Serratia* genus. The identified *Serratia* species included *Serratia grimesii* KY-SS7 (GenBank: PV459074.1) and KY-SS9 (GenBank: PV457913.1), *Serratia liquefaciens* DKY-SS10 (GenBank: PV458288.1) and DKY-SS3 (GenBank: PV459209.1), *Serratia proteamaculans* DKY-SS16 (GenBank: PV459206.1) and DKY-SS17 (GenBank: PV459208.1), as well as one unidentified *Serratia* sp., DKY-SS11 (GenBank: PV458349.1), which has not yet been classified at the species level. All isolates were submitted to the GenBank database.

3.2. Antibiotic susceptibility profiles

It was determined that the antibiogram zone diameters obtained for the *Escherichia coli* ATCC® 25922 reference strain were within CLSI standards (Table 1). The antibiotic susceptibilities of *Serratia grimesii*, *Serratia liquefaciens*, *Serratia proteamaculans*, and *Serratia* sp. isolates that could not be identified at the species level were evaluated within the scope of the study, and the results are presented in Table 1.

When the antibiotic susceptibility profiles were examined, it was determined that all *Serratia* species included in the study were resistant to the antibiotic cephalothin (KF). The *S. liquefaciens* DKY-SS10 strain showed

intermediate susceptibility to the antibiotic nalidixic acid (NA). However, *S. liquefaciens* DKY-SS3 strain was found to be resistant to a total of four antibiotics, three of which were beta-lactam antibiotics (cephalothin, ampicillin, and amoxicillin-clavulanic acid) and one was the folic acid synthesis inhibitor trimethoprim-sulfamethoxazole.

Table 1. Antibiotic susceptibility profiles of *Serratia* strains and *Escherichia coli* ATCC® 25922

Antibiotics	*Serratia grimesii KY-SS7*	*Serratia grimesii KY-SS9*	*Serratia liquefaciens DKY-SS3*	*Serratia liquefaciens DKY-SS10*	*Serratia proteamaculans DKY-SS16*	*Serratia proteamaculans DKY-SS17*	*Serratia* sp. DKY-SS11	*Escherichia* coli* ATCC® 25922
AMC	S	S	R	S	S	S	R	S
KF	R	R	R	R	R	R	R	S
IPM	S	S	S	S	S	S	S	S
DO	S	S	S	S	S	S	S	S
CIP	S	S	S	S	S	S	S	S
CRO	S	S	S	S	S	S	S	S
CTX	S	S	S	S	S	S	S	S
SXT	S	S	R	S	S	S	S	S
AM	S	S	R	R	S	S	S	S
C	S	S	S	S	S	S	S	S
MEM	S	S	S	S	S	S	S	S
NA	S	S	S	I	S	S	S	S
CN	S	S	S	S	S	S	S	S
LEV	S	S	S	S	S	S	S	S

AMC: Amoxicillin-clavulanic acid, KF: Cephalothin, IPM: Imipenem, DO: Doxycycline, CIP: Ciprofloxacin, CRO: Ceftriaxone, CTX: Cefotaxime, SXT: Trimethoprim-sulfamethoxazole, AM: Ampicillin, C: Chloramphenicol, MEM: Meropenem, NA: Nalidixic acid, CN: Gentamicin, LEV: Levofloxacin, S: Susceptible, I: Intermediate, R: Resistant.

3.3. Chemical composition of *P. betle* leaf essential oil

In this study, 37 components of the *P. betle* leaf essential oil were identified by GC-MS analysis (Table 2), a representative chromatogram is shown in Figure 1. The most abundant compounds were chavicol (31.47%), phenol, 2-methoxy-3-(2-propenyl)- (21.20%), caryophyllene (10.19%), phenol, 4-(2-propenyl)-, acetate (9.07%), and estragole (5.66%). These five compounds accounted for 77.59% of the total oil content.

Table 2. Chemical composition of *P. betle* leaf essential oil obtained by GC-MS analysis

Peak	Retention Time	Area%	Name
1	10.111	0.21	Camphene
2	13.464	0.51	Eucalyptol
3	14.252	0.09	1,3,6-Octatriene, 3,7-dimethyl-, (E)-
4	20.183	5.66	Estragole
5	23.007	31.47	Chavicol
6	25.542	9.07	Phenol, 4-(2-propenyl)-, acetate
7	26.389	0.10	alpha-Ylangene
8	26.564	21.20	Phenol, 2-methoxy-3-(2-propenyl)-
9	27.124	0.60	Beta- elemene
10	27.595	0.26	Benzene, 1,2-dimethoxy-4-(2-propenyl)-
11	28.065	10.19	Caryophyllene
12	28.361	0.28	Germacrene-D
13	28.710	0.68	Aromadendrene
14	29.201	1.15	alpha-Humulene
15	29.425	0.08	Alloaromadendrene
16	29.829	0.26	Cadina-1(6),4-diene
17	29.947	2.91	Gamma- Cadinene
18	30.049	0.30	alpha-Muurolene
19	30.290	0.24	beta-Selinene
20	30.380	0.12	Naphthalene, 1,2,4a,5,8,8a-hexahydro-4,7-dimethyl-1-(1-methylethyl)-
21	30.552	1.54	Viridiflorene
22	30.714	0.85	alpha-Muurolene
23	31.178	0.76	gamma-Cadinene
24	31.445	2.97	delta-Cadinene
25	31.596	3.57	Phenol, 2-methoxy-4-(2-propenyl)-, acetate
26	31.751	0.28	Naphthalene, 1,2,3,4,4a,7-hexahydro-1,6-dimethyl-4-(1-methylethyl)-
27	32.102	0.22	.alpha.-Calacorene
28	32.929	0.08	Viridiflorol
29	33.365	0.35	Caryophyllene oxide
30	33.463	0.57	(-)-Globulol
31	33.736	0.46	1H-Cycloprop[e]azulen-4-ol, decahydro-1,1,4,7-tetramethyl-
32	34.006	0.24	2-Naphthalenemethanol, 2,3,4,4a,5,6,7,8-octahydro-.alpha.,.alpha.,4a,8-tetramethyl-
33	34.484	0.21	Humulane-1,6-dien-3-ol
34	34.752	0.23	Di-epi-.alpha.-cedrene-(I)
35	35.194	1.09	tau-Cadinol
36	35.316	0.23	alpha-,epi-Muurolol
37	35.597	0.94	alpha-Cadinol

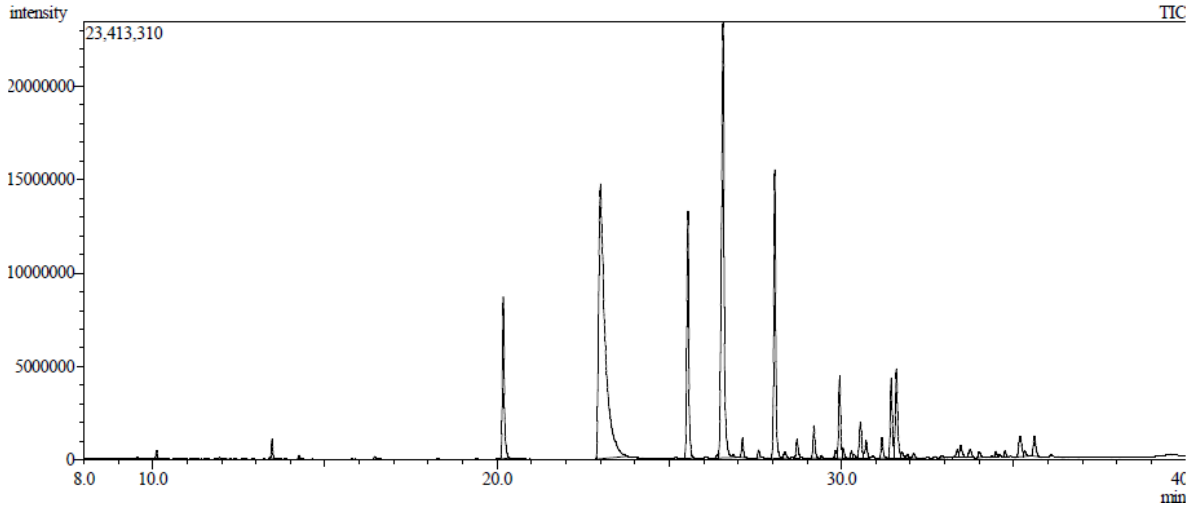

Figure 1. GC-MS chromatogram of *Piper betle* leaf essential oil showing the identified major and minor components

3.4. Antibacterial activity of *P. betle* essential oil against *Serratia* isolates

P. betle leaf essential oil showed a strong inhibitory effect on all tested *Serratia* strains. Inhibition zone diameters ranged from 20 mm to 28 mm (Table 3). *P. betle* leaf essential oil showed a strong antibacterial effect on all tested *Serratia* strains. Minimum inhibitory concentration (MIC) values were determined to be 0.078% for most strains; *Serratia* sp. DKY-SS11 strain was the most susceptible strain with the lowest MIC and MBC values (0.039%). The highest MBC values (0.156%) were observed in *S. liquefaciens* DKY-SS3 and *S. proteamaculans* DKY-SS16 strains.

Table 3. Antibacterial effect of *P. betle* essential oil on *Serratia* strains

Bacteria	Mean Zone diameter n)* ± Standard Deviation	MIC (%)	MBC (%)	Result
Serratia grimesii KY-SS7	20.33±0.58	0.078	0.078	SI
Serratia grimesii KY-SS9	26.33±0.58	0.078	0.078	SI
Serratia liquefaciens DKY-SS3	20.33±0.58	0.078	0.156	SI
Serratia liquefaciens DKY-SS10	28.33±1.00	0.078	0.078	SI
Serratia proteamaculans DKY-SS16	20.33±0.58	0.078	0.156	SI
Serratia proteamaculans DKY-SS17	24±1.00	0.078	0.078	SI
Serratia sp. DKY-SS11	24.67±1.00	0.039	0.039	SI

* measured including 6 mm disk. SI: Strong inhibitory

4. Discussion

In this study, the antibiotic resistance profiles of different *Serratia* species isolated from seafood salads available in the market in Türkiye were analyzed. It is noteworthy that 7 (58%) of the 12 isolates belonging to the Enterobacteriaceae family belonged to the *Serratia* genus. This situation can be explained by the fact that Serratia species become dominant in the microbial composition in cold-stored food products, especially when temperature fluctuations occur or when MAP conditions are applied. Indeed, Chen et al. (2024) reported that temperature fluctuations (4–8 °C) increased the relative abundance of *Serratia* in groupers' fillets and that this bacterium became an important factor causing spoilage under MAP. Similarly, *Serratia* was found to be the dominant species at the end of the shelf life in sea bream fillets stored at 12 °C under MAP (Anagnostopoulos et al., 2023). These findings reveal that facultative anaerobes such as *Serratia* may have a more advantageous position, especially in seafood products where the cold chain is disrupted or exposed to temperature fluctuations, and support the *Serratia* dominance observed in our study.

In this study, it was determined that some *Serratia* isolates were resistant to antibiotics such as cephalothin,

ampicillin, amoxicillin-clavulanic acid and/or trimethoprim-sulfamethoxazole. Similarly, in the study conducted by Sánchez-Pérez et al. (2025), it was reported that *Serratia marcescens* isolates were intrinsically resistant to ampicillin, cephalothin, tetracycline and polymyxin B. In the comparative analysis of 32 *Serratia* genomes performed by Sandner-Miranda et al. (2018), it was revealed that environmental strains were a larger reservoir of antibiotic resistance genes (ARGs) than thought and that these strains carried a similar number of resistance genes as clinical isolates. This suggests that some resistance genes in the *Serratia* genus are naturally encoded genomically. In support of this hypothesis, Krahulcová et al. (2023) observed intrinsic resistance to ampicillin in *S. liquefaciens* strains isolated from tuna sushi samples obtained from markets in Slovakia.

In addition, Sánchez-Pérez et al. (2025) reported that some clinical *S. marcescens* strains were phylogenetically closer to environmental strains, and that these strains carried resistance genes to antibiotics such as chloramphenicol, fosfomycin, and tetracycline. As a result, the potential of such *Serratia* isolates to reach humans through the food chain poses a significant risk to public health. Therefore, increasing food safety and storage time by using alternative natural additives has become one of the priority approaches today. In this context, in our study, the antibacterial potential of *P. betle* leaf essential oil against seafood-derived *Serratia* isolates was evaluated by both disk diffusion and microdilution methods.

First, the chemical composition of *P. betle* essential oil was analyzed using the GC-MS technique in order to better understand its potential antibacterial effect. As a result of the analysis, chavicol, phenol, 2-methoxy-3-(2-propenyl)-, caryophyllene, phenol, 4-(2-propenyl)-, acetate and estragole stood out as the most abundant components. It was observed that these findings were reported similarly in different studies in the literature (Sarkar & Sawardekar, 2022; Tran et al., 2023). In this study, it was revealed that *P. betle* leaf essential oil showed a strong antibacterial effect on seafood-derived *Serratia* strains, both with inhibition zone diameters and low MIC and MBK values. Similarly, a strong inhibitory effect of *P. betle* leaf ethanolic extract on *S. marcescens* was reported (Valle et al., 2016).

5. Conclusion

This study highlights the potential risk of foodborne environmental strains to public health by revealing the presence of *Serratia* species in ready-to-eat seafood salads sold in Türkiye and the antibiotic resistance profiles of these isolates. The resistance observed, especially against antibiotics such as cephalothin, ampicillin, and trimethoprim-sulfamethoxazole, suggests that these species may be related to intrinsic resistance mechanisms. This situation shows that temperature fluctuations that may occur under cold chain conditions may cause facultative anaerobic bacteria such as *Serratia* to become dominant in the microbial community and reveal the importance of monitoring contamination sources. In addition, in line with the need for alternative preservative strategies, it was determined that the essential oil of *P. betle* leaf tested in the study showed strong antibacterial activity against all *Serratia* strains. In future studies, it is recommended that the applicability of this oil in seafood products be evaluated and product development and shelf life tests be performed.

6. Compliance with Ethical Standard

a) Author Contributions

1. DKY.: Conceptualization, Investigation, Validation, Data Curation, Formal analysis, Methodology, Writing—review and editing; 2. MA.: Methodology, Investigation; The published version of the manuscript has been read and approved by both authors.

b) Conflict of Interests

There is no conflict of interest, according to the authors.

c) Statement on the Welfare of Animals

Not relevant

d) Statement of Human Rights

There are no human subjects in this study.

e) Funding

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