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ARAŞTIRMA MAKALESİ **RESEARCH PAPER**

Determination of Antimicrobial Activity and MIC Value of Tannic Acid Against Four Different Fish Pathogens

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Abstract: In this study, the antimicrobial susceptibility of Silvafeed TSP (tannic acid) obtained from chestnut tree using natural extraction method and used as a feed sweetener in aquaculture against some fish pathogens was investigated. Antimicrobial susceptibility of different concentrations of tannic acid was determined against fish pathogens that cause significant economic losses by bacteria such as *Aeromonas hydrophila, Pseudomonas fluorescens, Yersinia ruckeri* and *Vibrio anguillarum*. According to the antimicrobial test results, it was determined that all isolates to tested antibiotics were resistant to ampicillin; however, all isolates were susceptible to other antibiotics except for the *P. fluorescens* isolate. It was determined that the *P. fluorescens* isolate was resistant to other antibiotics except gentamicin and doxycycline. According to the antimicrobial susceptibility test results of tannic acid against *E. coli* and four different fish pathogens, *P. fluorescens* and *V. anguillarum* were found to be sensitive above 125 µg/ml concentrations. In the same study, *E. coli, A. hydrophila,* and *Y. ruckeri* were found to be sensitive above 250 µg/ml concentrations. It was determined that *P. fluorescens* and *V. anguillarum* were resistant to tannic acid concentrations below 62.5 µg/ml. Moreover, *E. coli, A. hydrophila,* and *Y. ruckeri* were resistant to tannic acid concentrations below 125 µg/ml. The MIC value of tannic acid was determined in tubes with a concentration of 187.5 µg/ml for *E. coli*, 125 µg/ml for *Y. ruckeri*, 31.25 µg/ml for *P. fluorescens*, and 375 µg/ml for *V. anguillarum*.

Keywords: Antimicrobial susceptibility, aquaculture, fish pathogens, tannic acid, treatment.

Tannik Asidin Dört Farklı Balık Patojenine Karşı Antimikrobiyal Aktivitesinin ve MİK Değerinin Belirlenmesi

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Öz: Bu çalışmada, kestane ağacından doğal ekstraksiyon yöntemi ile elde edilen ve su ürünleri yetiştiriciliğinde yem tatlandırıcı olarak kullanılan Silvafeed TSP'nin (tannik asit) bazı balık patojenlerine karşı antimikrobiyal duyarlılığı araştırılmıştır. *Aeromonas hydrophila, Pseudomonas fluorescens, Yersinia ruckeri* ve *Vibrio anguillarum* gibi bakteriler tarafından önemli ekonomik kayıplara neden olan balık patojenlerine karşı farklı konsantrasyonlarda tannik asidin antimikrobiyal duyarlılığı belirlendi. Antimikrobiyal test sonuçlarına göre, test edilen antibiyotiklere karşı izolatların tamamının ampisiline dirençli olduğu, ancak *P. fluorescens* izolatı hariç diğer antibiyotiklere duyarlı olduğu belirlendi. *P. fluorescens* izolatının gentamisin ve doksisiklin hariç diğer antibiyotiklere dirençli olduğu belirlendi. Tannik asidin *E. coli* ve dört farklı balık patojeni olan *P. fluorescens* ve *V. anguillarum*'a karşı antimikrobiyal duyarlılık test sonuçlarına göre, 125 µg/ml konsantrasyonun üstünde duyarlı olduğu bulundu. Aynı çalışmada *E. coli, A. hydrophila* ve *Y. ruckeri*'nin 250 µg/ml konsantrasyonun üzerinde duyarlı olduğu bulunmuştur. *P. fluorescens* ve *V. anguillarum*'un 62,5 µg/ml'nin altındaki tannik asit konsantrasyonlarına dirençli olduğu belirlendi. Ayrıca, *E. coli, A. hydrophila* ve *Y. ruckeri*'nin 125 µg/ml'nin altındaki tannik asit konsantrasyonlarına dirençli olduğu belirlendi. Tannik asidin MİK değeri, E. coli için 187,5 µg/ml, Y. ruckeri için 125 µg/ml, P. fluorescens için 31,25 µg/ml ve V. anguillarum için 375 µg/ml konsantrasyona sahip tüplerde belirlendi.

Anahtar kelimeler: Antimikrobiyal duyarlılık, balık patojenleri, su ürünleri yetiştiriciliği, tannik asit, tedavi.

INTRODUCTION

In rainbow trout farming, fish disease problems are frequently encountered due to the increasing stock density in fish ponds in order to meet increasing consumer demands. Intensive fish stocking in fish farms often leads to the emergence of stress-related diseases. The absence of early diagnosis and treatment of these diseases cause great economic losses like weight loss and mass deaths. This case significantly affects sustainable production in aquaculture. Antibiotics are used to treat bacterial agents because they are generally the most effective and cheapest according to the antimicrobial test results. In recent years, it has been reported that bacteria have become resistant to antibiotics used in aquaculture and have caused residual problems in terms of human health (Balta, 1999; Balta & Çağırgan, 2007; 2019). Disease problems are encountered in rainbow trout farms in terrestrial and marine environments in the Black Sea region due to intensive production and environmental stress. In particular, furunculosis, yersiniosis, vibriosis and pseudomonas disease agents have been isolated in rainbow trout in net cages in the Black Sea (Altinok et al., 2007; Balta, 2016; 2020; Balta & Dengiz Balta, 2016; 2017; Balta et al., 2016; Balta & Yılmaz, 2019; Kacar & Balta, 2017). It has been reported that they are water-soluble polyphenolic compounds that group chemicals with important biological properties such as tannins (tannic acids), antimicrobial, anti-inflammatory, anti-viral, antioxidant, and anti-parasitic activities (Alavinia et al., 2018; Pérez-Fonseca et al., 2016). It was reported that polyphenol tannic acid (TA) was 100% effective on *Ichthyophthirius multifiliis* theronts and tomonts by damaging the plasma membrane depending on dose and time. It has been notified that tannins are commonly found in different parts of the bark, fruit, leaves, and roots of various plants (EL-Hefny et al., 2017). There are two different types of tannins that can be hydrolyzed and condensed. It has been reported that tannins have major functions such as binding and precipitating proteins and other macromolecules (Brooker et al., 1994). The European Community banned the use of antibiotics as growth promoters in animal nutrition in 2006. As a result, many approaches have been tried to control or prevent subclinical diseases in animals as well as to improve growth performance and maximize economic sustainability (Schiavone et al., 2008). In the study, tannic acid, which is a natural feed additive of plant origin (chestnut tree), was considered an alternative to antibiotics in order to eliminate these problems and raise fish in a healthy way. The tannic acid is a tannin compound with a sour taste, in brown powder form, with a minimum of 75% polyphenol content, pH 3-5 in its 10% solution and a maximum of 8% humidity (Balta, & Tekin, 2021). It has been shown that tannin has an inhibitory effect on microorganisms by decreasing enzyme activity, dysfunction of the cell membrane, as well as deprivation of substrate, metal ions and minerals (Cipriano-Salazara et al., 2018; Goel et al., 2005).

In this study, it was aimed to determine the antimicrobial activity of silvafeed (tannic acid) against *Aeromonas hydrophila*, *Pseudomonas fluorescens*, *Yersinia ruckeri*, and *Vibrio anguillarum* isolated from disease cases in rainbow freshwater trout farms and sea trout farms in the Black Sea region.

MATERIAL AND METHOD

Silvafeed TSP (Tannic Acid): Silvafeed TSP used in this study was obtained from Profeed Food and Chemical Substances Industry and Trade Ltd. Şti. Silvafeed TSP is marketed as a commercial product in the form of a water-soluble powder obtained using natural extraction processes from plants. To determine the antimicrobial activity of silvafeed, 0.1 g and 0.2 g were weighed on a precision balance (0.0001 g) and dissolved in 100 ml of a suitable solvent. Ten-fold dilutions of Silvafeed were prepared in 20 ml glass bottles.

Test Microorganisms: In the study, four different Gram-negative bacteria isolated from disease outbreaks in rainbow trout farms in the Eastern Black Sea Region were used. *A. hydrophila (*A08, A25, A53, A61) (Balta, 2020), *P. fluorescens* (P08, P29, P57, P73) (Kacar & Balta, 2017), *V. anguillarum* (V87, V140, V823, V976) (Balta, 2016; Balta & Dengiz Balta, 2017; Balta & Yılmaz, 2019), and *Y. ruckeri* (Y15, Y25, Y35, Y61) isolates (Balta et al., 2010; 2016), which were identified in previous studies and stocked at -80°C in a medium containing 1.5% glycerol, were grown on Tryptic Soy Broth (TSB). The effectiveness of dilutions of tannic acid in different concentrates was determined by preliminary studies on the diameters of the inhibition zones formed against the quality control strainof *E. coli* (ATCC 25922). Zone diameters formed by fish pathogens were compared with standard of quality control strain *E. coli* zone diameters. The bacterial density used in the study was adjusted according to McFarland 0.5 density. The bacterial isolates and their origins tested in the study are given in Table 1.

Chemicals: Tannic acid was weighed in three different amounts (0.1 g, 0.2 g and 0.3 g) on a precision balance. Tannic acid was dissolved in a 100 ml flask with 2% methanol containing 0.1M HCl. A magnetic bar was placed in the flasks containing tannic acid and homogenized with a magnetic stirrer. Tannic acid solutions were sterilized with sterile 0.45 µm membrane filters

(Merck-Millipore) with injector attachment. Tannic acid solutions were divided into sterile 20 ml bottles of 5 ml each and stored at -20°C. During the study, tannic acid stock solutions were removed from the deep freezer at -20°C. It was melted at room temperature. Tannic acid was diluted from the main stock solution with the help of sterile distilled water and different concentrations of tannic acid were prepared distinct solutions, which are given in Table 2. Antibiotic discs (Bioanalysis) used in the antimicrobial tests were purchased from a commercial company.

Table 1. The location and origin of bacterial isolates used in this study.

No	Bacteria species	Origins	Location
E1	*E. coli*	ATCC 25922	American Type Culture
A08	*A. hydrophila*	Kidney of rainbow trout	Fish farm / Artvin
A25	*A. hydrophila*	Kidney of rainbow trout	Fish farm / Erzurum
A53	*A. hydrophila*	Kidney of rainbow trout	Sea Fish farm / Rize
A61	*A. hydrophila*	Kidney of rainbow trout	Sea Fish farm / Trabzon
P08	*P. fluorescens*	Kidney of rainbow trout	Fish farm / Artvin
P29	*P. fluorescens*	Kidney of rainbow trout	Fish farm / Gümüşhane
P53	*P. fluorescens*	Kidney of rainbow trout	Fish farm / Rize
P58	*P. fluorescens*	Kidney of rainbow trout	Fish farm / Sivas
V08	*V. anguillarum*	Kidney of rainbow trout	Sea Fish farm / Artvin
V52	*V. anguillarum*	Kidney of rainbow trout	Sea Fish farm / Ordu
V53	*V. anguillarum*	Kidney of rainbow trout	Sea Fish farm / Trabzon
V61	*V. anguillarum*	Kidney of rainbow trout	Sea Fish farm / Rize
Y08	*Y. ruckeri*	Kidney of rainbow trout	Fish farm / Artvin
Y25	*Y. ruckeri*	Kidney of rainbow trout	Fish farm / Erzurum
Y28	*Y. ruckeri*	Kidney of rainbow trout	Fish farm / Giresun
Y61	*Y. ruckeri*	Kidney of rainbow trout	Sea Fish farm / Trabzon

Table 2. Concentrations of two-fold serial dilutions of tannic acid.

Tubes	Two-fold serial dilutions of tannic acid	Concentration
1.	1 ml→ 2 mg(=2000 µg)	2000 µg TA
2.	1 ml 2000 µg TA + 1 ml sterile water	1000 µg TA
3.	1 ml 1000 µg TA + 1 ml sterile water	500 µg TA
4.	1 ml 500 µg TA + 1 ml sterile water	250 µg TA
5.	1 ml 250 µg TA + 1 ml sterile water	125 µg TA
6.	1 ml 125 µg TA + 1 ml sterile water	62,5 µg TA
7.	1 ml 62,5 µg TA + 1 ml sterile water	31,25 µg TA
8.	1 ml 31,25 µg TA + 1 ml sterile water	15,625 µg TA
9.	1 ml 15,625 µg TA + 1 ml sterile water	7,8125 µg TA
10	1 ml 7,8125 µg TA + 1 ml sterile water	3,90625 µg TA
11.	0,1 M HCl + methanol	Control

Media Preparation: In this study, the method used by Arret et al. in 1971 was modified to determine the antimicrobial susceptibility of tannic acid (Balta, 1999; Balta & Çagırgan, 2007; Balta & Çagırgan, 2010). Briefly, Mueller Hinton Agar (MHA) instead of Antibiotic Medium No: 2 was used for antimicrobial testing. Zone diameters formed by tannic acid for five different bacteria were measured using a digital compass (Mitutoyo). MHA and MHA with 1.5% added salt were prepared for the microorganisms to be tested. The sensitivity of tannic acid to *V. anguillarum* was performed in saline MHA. The sensitivity of other microorganisms was tested in MHA. Briefly, four petri dishes were placed on A4 paper and four circles were drawn. Then, with the help of a ruler and digital caliper, a template was prepared by marking the places where six porcelain beads would be placed at equal distances from each other. Six sterile porcelain beads were placed evenly in the petri dish, then poured and cooled to 50°C sterile MHA into petri dish. After the Mueller Hinton agars were frozen, the porcelain beads were removed with the help of sterile forceps and kept in the refrigerator at +4°C for a maximum of 10 days until used.

Disk Diffusion Test: In addition, the sensitivity of the same bacterial strains to eight different antibiotics was determined. Disc diffusion method was used to determine the antibiotic susceptibility of these microorganisms (CLSI, 2006). For this purpose, overnight cultures of each bacterial isolate and quality control strain used in the study were homogenized in tubes containing 2 ml FTS and adjusted to Mc Farland No 0.5 density. Then, 0.1 ml of the prepared bacterial suspensions were taken and inoculated into MHA. Antibiotic discs were placed on the MHA with the help of an automatic dispenser. In the study, 8 different antibiotics, namely ampicillin (10 µg), gentamicin (10 µg), doxycycline (30 µg), enrofloxacin (5 µg), oxytetracycline (30 µg), sulfamethoxazole (100 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg) disk (Bioanalysis) were used. MHA were incubated for 24 hours at 28°C (CLSI, 2006). After incubation, the diameters of the inhibition zones where the bacteria formed around the antibiotic discs did not grow were measured with the help of a digital caliper. Standard zone diameters formed by different antibiotics are given in Table 3.

Table 3. Zone diameter standards of different antibiotics.

Antimicrobial agents	Measured inhibition zone diameter (mm)			
	R	I	S	Literature
AM-10 µg	≤ 13	14-16	≥ 17	CLSI, 2014
CN-10 µg	≤ 12	13-14	≥ 15	CLSI, 2014
DO-30 µg	≤ 10	11-13	≥ 14	CLSI, 2013
ENR-5 µg	≤ 16	17-20	≥ 21	CLSI, 2014
FFC-30 µg	≤ 14	15-18	≥ 19	CLSI, 2014
T-30 µg	≤ 14	15-18	≥ 19	CLSI, 2014
SMZ-100 µg	≤ 12	13-16	≥ 17	CLSI, 2014
SXT-25 µg	≤ 10	11-15	≥ 16	CLSI, 2014

AM: Ampicillin, CN: Gentamicin, DO: Doxycycline, ENR: Enrofloxacin, FFC: Florfenicol, T: Oxytetracycline, SMZ: Sulfamethoxazole, SXT: Trimethoprim/Sulfamethoxazole,
R: Resistant, I: Intermediate, S: Sensitive.

Determination of Antimicrobial Susceptibility of Tannic Acid: This study was carried out to determine the antimicrobial susceptibility of five different Gram-negative bacterial isolates to tannic acid. Microorganisms to be tested were removed from the deep freezer at -80°C. *E. coli*, *A. hydrophila*, *P. fluorescens*, and *Y. ruckeri* were seeded into TSA. *V. anguillarum* was inoculated into TSA supplemented with 1.5% salt. The seeded media were incubated for 48 hours in a refrigerated incubator at 20°C. These media were incubated for 24 hours in a refrigerated incubator at 20°C. Pure bacterial colonies in fresh cultures were received by loop and adjusted to McFarland 0.5 turbidity in sterile 1.5 ml physiological saline (0.9% NaCl). The pre-prepared MHA with wells was removed from the refrigerator and dried in an incubator at 36°C for 30 minutes. 1.5 ml of bacterial suspension adjusted to McFarland 0.5 turbidity was poured onto dried MHA and spread over the medium for a period of 30 seconds by wrist movement. Excess bacterial suspensions were collected in a beaker to be sterilized in an autoclave. 100 µl of each of

the 10-fold dilutions prepared at different concentrations from the tannic acid stock solution was added to the well created in MHA using a micropipette. Each different concentration was studied in triplicate. MHA media with different concentrations of tannic acid were incubated for 24 hours in a cooling incubator at 28°C. The diameters of the zones where bacteria did not grow around each well were measured with the help of digital caliper (Mitutoyo) and recorded by taking the average of the zone diameters.

Determination of Minimal Inhibitory Concentration (MIC): While preparing the stock solution of tannic acid to be used in the test, 100 ml of methanol solution containing 2% HCl (Merck) was used. Tannic acid was weighed 0.2 and 0.3 gr on a precision balance and dissolved in a methanol solution containing 2% 0.1M HCl in a 100 ml flask. Tannic acid stock solutions were divided into sterile 20 ml bottles of 5 ml each and stored in a deep freezer at -20°C. Two-fold serial dilutions were performed in a previously sterilized tannic acid stock solution, Mueller Hinton Broth (MHB). Then, 10 µl of fresh overnight bacterial cultures grown in TSB (supplemented with 1.5% salt for *Vibrio*) at 25°C were added to each test tube except for the control group. All of the tubes were incubated at 25°C for 24 hours. MIC values were performed in duplicate for each of the different concentrations of tannic acid. The lowest concentration of the chemical substance in the tubes where bacteria did not grow was accepted as the MIC value (CLSI, 2015). Different concentrations of two-fold serial dilutions prepared in Mueller Hinton Broth to determine tannic acid MIC values are given in Table 6.

RESULTS

In this study, the sensitivity of five different bacterial isolates to silvafeed TSP (tannic acid), was determined. In the same study, tannic acid MIC values of five different bacterial isolates were determined.

Disk Diffusion Test: In this study, the zone diameters of five different bacteria against eight different antibiotics in MHA were measured by the agar disc diffusion method, and these values were compared with the standard zone diameter values in Table 3 to determine the antimicrobial sensitivity of five microorganisms. It was determined that *P. fluorescens*, one of the bacteria used in the study, was resistant to all other antimicrobials used in the study except gentamicin and doxycycline. *A. hydrophila*, *E. coli*, *V. anguillarum*, and *Y. ruckeri* were sensitive to other antibiotics except ampicillin. Sensitivity values of five microorganisms are given in Table 4.

Determination Antimicrobial Sensitivity of Tannic Acid with Agar Well Diffusion Testy: In this study, the antimicrobial activity of tannic acid at different concentrations was determined against five different microorganisms. While tannic acid was detected to be sensitive above 250 µg/ml against *E. coli*, *A. hydrophila*, and *Y. ruckeri* isolates, these values have been found to be sensitive over 125 µg/ml for *P. fluorescens* and *V. anguillarum*. The antimicrobial susceptibility of tannic acid (TA) against five different bacterial isolates is given in Table 5.

Table 4. Antimicrobial susceptibility of eight antibiotics against test microorganisms.

Bacterial isolates	Antibiotic Discs							
	AM	CN	DO	ENR	FFC	T	SMZ	STX
E01	R	S	S	S	S	S	S	S
A08	R	S	S	S	S	R	I	S
A25	R	S	S	S	S	R	I	S
A53	R	S	S	S	S	R	I	S
A61	R	S	S	S	S	R	S	S
P08	R	S	I	R	I	R	R	R
P29	R	S	I	R	R	R	R	R
P53	R	S	S	R	R	R	R	R
P58	R	S	S	R	R	R	R	R
V08	R	S	S	S	S	I	R	I
V52	R	S	S	S	S	I	I	S
V53	R	S	S	S	R	R	R	R
V61	R	S	S	S	S	S	S	S
Y15	R	S	S	S	R	I	R	I
Y25	R	S	S	S	R	R	R	I
Y28	R	S	S	S	I	R	I	S
Y61	R	S	S	S	I	I	I	S

E01: *E. coli*, *A. hydrophila* (A08, A25, A53 and A61), *P. fluorescens* (P08, P29, P57 and P73), *V. anguillarum* (V87, V140, V823 and V976) and *Y. ruckeri* Y15, Y25, Y35 and Y61),
AM (10 µg): Ampicillin, CN (10 µg): Gentamicin, DO (30 µg): Doxycycline, ENR (5 µg): Enrofloxacin, FFC (30 µg): Florfenicol, OTC (30 µg): Oxytetracycline, SMZ (100 µg): Sulfamethoxazole, STX (25 µg): Trimethoprim/Sulfamethoxazole,
R: Resistant, I: Intermediate, S: Sensitive

Table 5. Antimicrobial susceptibility of tannic acid (TA) to five different bacterial isolates.

Bacterial isolates	Tannic Acid Concentration (µg/ml)							
	2000	1500	1000	500	250	125	62,5	31,25
E01	S	S	S	S	S	R	R	R
A08	S	S	S	S	S	R	R	R
A25	S	S	S	S	S	R	R	R
A53	S	S	S	S	S	R	R	R
A61	S	S	S	S	S	R	R	R
P08	S	S	S	S	S	S	R	R
P29	S	S	S	S	S	S	R	R
P53	S	S	S	S	S	S	R	R
P58	S	S	S	S	S	S	R	R
V08	S	S	S	S	S	S	R	R
V52	S	S	S	S	S	S	R	R
V53	S	S	S	S	S	S	R	R
V61	S	S	S	S	S	S	R	R
Y15	S	S	S	S	S	R	R	R
Y25	S	S	S	S	S	R	R	R
Y28	S	S	S	S	S	R	R	R
Y61	S	S	S	S	S	R	R	R

E01: *E. coli*, *A. hydrophila* (A08, A25, A53 and A61), *P. fluoresens* (P08, P29, P53 and P58), *V. anguillarum* (V08, V52, V53 and V61) and *Y. ruckeri* Y15, Y25, Y28 and Y61),
R: Resistant, S: Sensitive.

Determination of Minimal Inhibitory Concentration (MIC): In this study, the MIC values of five different bacterial isolates were investigated against different concentrations of tannic acid (0.2 and 0.3 g stock). Although *A. hydrophila* did grow at a concentration of 187.5 µg/ml MIC value, no bacteria were observed in tubes with a concentration of 250 µg/ml. MIC value of 250 µg/ml was accepted as the first tube in which *A. hydrophila* did not grow in MHB tubes with tannic acid. It was determined that the MIC values for tannic acid at different concentrations with *E. coli* and *Y. ruckeri* were found in the tube with concentrations of 187.5 µg/ml and 125 µg/ml,

respectively. The MIC value of tannic acid was found in a tube with a concentration of 31.25 µg/ml for *P. fluorescens* and 375 µg/ml for *V. anguillarum*. No bacteria were added to the control group, but sterile 0.9% NaCl was added. In addition, the effectiveness of 2% methanol and 0.1M HCl used in the preparation of the main stock of tannic acid was determined by preliminary tests to have no effect on bacterial growth. The MIC values of tannic acid against isolates of five different bacterial species are given in Table 6.

Table 6. MIC values of tannic acid against isolates of five different bacterial species.

Bacterial isolates	Tannic Acid Concentration (µg/ml)															
	3000	1500	1000	750	500	375	250	187,5	125	93,75	62,5	46,875	31,25	23,44	15,625	Control
E01	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
A08	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
A25	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
A53	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
A61	-	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+
P08	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
P29	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
P53	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+
P58	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+	+
V08	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
V53	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
V53	-	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+
V61	-	-	-	-	-	-	+	+	+	+	+	+	+	+	+	+
Y15	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
Y25	-	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+
Y28	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+
Y61	-	-	-	-	-	-	-	-	-	-	+	+	+	+	+	+

E01: *E. coli*, *A. hydrophila* (A08, A25, A53 and A61), *P. fluorescens* (P08, P29, P53and P58), *V. anguillarum* (V08, V52, V53 and V61) and *Y. ruckeri* Y15, Y25, Y28 and Y61),
-: no bacterial growth, +: bacterial growth.

DISCUSSION AND CONCLUSION

Gram-negative bacterial fish pathogens cause mass mortality in fry and adult fish of cultured rainbow trout due to water temperature stress. Different antibiotics are used according to the antibiogram test results in the treatment of infections caused by *A. hydrophila*, *P. fluorescens*, *Y. ruckeri*, and *V. anguillarum* (Cagirgan & Tanrikul, 1998; Altinok et al., 2007; Balta et al., 2010; 2016; Balta & Dengiz Balta, 2016; 2017; 2019; Balta, 2020; Dincturk & Tanrikul, 2021).

It was reported that the usage of random antibiotics for the treatment of diseases causes some problems in aquaculture. The short-term (biochemical) and persistent (genetic) resistance to these antibiotics are formed when antibiotics are not used at the appropriate dose and duration in the treatment of bacterial infections (Balta, 1999; Balta & Çağırgan, 2007; Balta & Çağırgan, 2010). It causes failure in the use of the same antibiotics in the treatment of repeated infections. Moreover, the use of antibiotics causes residual problems in fish meat as well as chemical pollution in the aquatic environment, changes the benthic fauna, and prevents sustainable production (Çagırgan & Tanrikul, 1998; Balta, 1999; Balta & Çağırgan, 2007; Balta & Çağırgan, 2010; Balta, 2016; Balta & Dengiz Balta, 2019; Balta & Yılmaz, 2019; Onuk et al., 2015; 2017; Duman et al., 2017; Dincturk & Tanrikul, 2021).

Tannic acid is a polyphenolic water-soluble compound obtained from different parts of plants such as stems and leaves. Tannins are reported to have important biological properties such as antimicrobial, anti-inflammatory, antiviral, antioxidant, and anti-parasitic activities (Aydın & Üstün, 2007; Cipriano-Salazara et al., 2018; Kaczmarek, 2020). Since tannic acid obtained from plants is a natural substance, its usage in the treatment of diseases caused by bacteria has been reported to be a privilege. This study aimed to determine the effectiveness of tannic acid against four different bacterial species that cause disease in fish. The use of tannic acid in the treatment of fish pathogens will support organic aquaculture. In addition, using tannic acid will eliminate the residual problems of chemotherapeutics, reduce environmental pollution, eliminate its negative effects on human health, and facilitate the export of fish abroad. Using tannic acid may prevent possible carcinogenic and mutagenic effects on humans due to fish antibiotic residues. The antimicrobial sensitivities of tannic acid were determined to be effective at different concentrations for each bacterial species by disc diffusion and MIC tests against Gram-negative bacteria. Studies on fish pathogens of tannic acid are quite limited. It has been reported that its antioxidant activity, effect on intestine movement and effects on intestinal microflora of tannic acid have been studied by different researchers (Sell et al., 1985; Yılmaz & Romeo, 2004; Schiavone et al., 2008). Thanks to its flavoring properties, tannic acid has been reported to increase feed intake and reduce feed stress in poultry as a feed additive (Zoccarato et al., 2006; Schiavone et al., 2008). It balances the microflora by changing the microflora preference thanks to the competitive exclusion mechanism (Schiavone et al., 2008). It increases animal welfare and reproductive performance while it decreases the use of antibiotics and the fish mortality. It also has a growth performance improvement effect as it suppresses the increase of pathogenic bacteria and toxin formation (Zoccarato et al., 2006; Zoccarato et al., 2008). It has been reported that when fish infected with *V. harveyi* are fed with plant extracts from plants, they gain weight due to increased appetite and significantly increases the amount of white blood cells, red blood cells, hemoglobin, hematocrit, lymphocytes, and monocytes (Harikrishnan et al., 2012). A

study was carried out to investigate the effects of different concentrations (10, 20 and 30 ppm) on the immune system of the extract prepared from the medicinal plant *Ocimum sanctum* in common carp (*Cyprinus carpio*) fingerlings inoculated against heat-killed *A. hydrophila*. In the study, it was reported that maximum antibody response and more phagocytic activity were detected in the low-concentration group (10 ppm) compared to the control and other experimental groups (20 and 30 ppm) (Pavaraj et al., 2011). In a study investigating the effects of extracts and mixtures of three different (*Crataegi fructus* (Cf), *Artemisia capillarie*s and *Cnidium officinale*) medicinal plants on the growth of red sea bream (Coral) larvae and against *V. anguillarum*, it was determined that the number of *Vibrio* decreased in rotifers fed with these plant extracts. It was reported that rotifers fed with Cf extract grew statistically more than the other groups. In the same study, it was reported that Cf and these plant mixtures inhibited the growth of five infectious bacterial isolates, including *Aeromonas, Edwasiella, Photobacterium, Pseudomonas* and *Vibrio* genera, in rotifers fed with methanol extracts. The survival rate of the fish larvae was found to be considerably higher in the challenge (protective effectiveness) test performed with *V. anguillarum* compared to the control group by feeding the plant extract mixture. These results reported that sap-fed herb rotifers are beneficial for increasing growth and resistance to *V. anguillarum* in sea bream larvae and provide a new technology for the sustainable production of disease-resistant offspring (Takaoka et al., 2011). Six different concentrations of Silvafeed TSP (0.05%, 0.01, 0.02, 0.04, 0.08 and 0.1) using the Kirby-Bauer disc diffusion method antimicrobial activity have been tested against important fish pathogens, *A. hydrophila, A. salmonicida, A. bestiarum, A. sobria, L. anguillarum, Lactococcus garvieae, P. fluorescens, P. anguilliseptica, Y. ruckeri,* and *Vagococcus salmoninarum*. Although all concentrations of Silvafeed TSP are susceptible to *L. anguillarum* and *Y. ruckeri*, it has been reported to be resistant to *L. garvieae* and *V. salmoninarum*. *Aeromonas* species (except *A. bestiarum*, 0.05%) are susceptible, *P. fluorescens* and *P. anguilliseptica* have been found to be intermediate sensitive in the first two concentrations but sensitive in the other four concentrations (Tanrikul & Dincturk, 2017). In a study on the effects of phytochemicals, oxytetracycline and enrofloxacin antibiotics have been determined to be resistant to *Y. ruckeri*. However, *Moringa oleifera* and *Sorbus domestica* plant extracts inhibited the growth of bacteria by 40-50% in measurements made in a liquid medium (TSB); their effects have been less in a solid medium (MHA) (Onalan & Çevik, 2020).

At the end of this study, it was thought that Silvafeed TSP could be effective in the treatment of Gram-negative bacterial fish pathogens, and it would be beneficial to add it to the feeds as a feed additive for preventive purposes before the disease occurs.

CONFLICT OF INTEREST

The author declares no conflict of interest.

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