

## Comparison of Antimicrobial Activities of *Sparus aurata* Skin and Mucus Extracts with *Laurencia papillosa* and *Carollina officinalis* Algae Dry Extracts

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### ABSTRACT

The present study aimed to determine the antimicrobial activity of methanol, acetone, ethanol, or heptane extracts from *L. papillosa* or *C. officinalis* with the skin and mucus extracts from *S. aurata*. The inhibition zone (IZ) and minimum inhibitory concentration (MIC) of the extracts against *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Enterococcus faecalis*, *Candida tropicalis*, and *C. parapsilosis* were determined by well diffusion and spectrophotometric broth microdilution methods, respectively. The highest antimicrobial activity of *L. papillosa* was against *C. tropicalis* with 4.98 mm, and the highest activity of *C. officinalis* was against *E. faecalis* with 7.84 mm. Among *S. aurata* mucus and skin extracts, the highest activity was on *C. parapsilosis* with 13.82 mm. The antimicrobial effect of *S. aurata* mucus extract on *E. faecalis* was found to be almost the same as *L. papillosa* extracts (6.14 mm and 6.43 mm). The inhibitions of *S. aurata* aqueous phase extract on *K. pneumoniae* and *C. parapsilosis* (7.09 mm) were much greater than the effects of *L. papillosa* and *C. officinalis* extraction. That *S. aurata* mucus and skin extracts were very effective, especially on *K. pneumoniae*, *A. baumannii* and *Candida* sp., was showed for the first time with this study. As a result, *S. aurata* mucus content is more effective on *K. pneumoniae* and *C. parapsilosis* than the phenolic content of both algae.

**KEYWORDS:** *Sparus aurata*, *Laurencia papillosa*, *Carollina officinalis*, Antimicrobial activity

**How to cite this article:** Erdoğan-Eliuz, E., Bakan, M., Ayas, D. (2024) Comparison of Antimicrobial Activities of *Sparus aurata* Skin and Mucus Extracts with *Laurencia papillosa* and *Carollina officinalis* Algae Dry Extracts. *MedFAR.*, 7(1):1-11

## 1. Introduction

Algae are of great importance in that they meet the nutritional needs of many living creatures, produce two-thirds of the world's photosynthetic carbon needs, contribute to the oxygen production in the atmosphere by 70-90%, and constitute approximately 90% of marine plants (Özdemir & Erkmén, 2013; Chapman, 2013; Baytaşoğlu & Başusta, 2015; Akyıl et al., 2016). Studies show that algae are rich in protein, fat, sterol, and water-soluble fiber content and have a high nutritional value in terms of minerals such as magnesium, potassium, and zinc (El-Sheekh et al., 2006; Alçay et al., 2017; Durmaz et al., 2002). Algae are the most encouraged biological resources in medicine, pharmacy, and cosmetics industries, agriculture, fertilizer production, and biodiesel production. In particular, it is widely used in the food industry as a gelling and thickening agent, such as agar-agar, alginate, and carrageenan (Kaba & Çağlak, 2006; Polat & Özoğul 2008; Carvalho et al., 2011). In addition, agar obtained from species belonging to the Rhodophyceae (Red algae) family is very important in bacteriology and biomedical studies (Baytaşoğlu & Başusta, 2015). It is known that the natural metabolites contained in algae are effective in defense against various pathogens (Kavita et al., 2014). As a result of studies on algae, many studies are determining their antimicrobial, cytotoxic, antimitogenic, anticancer, and antitumoral activities (Kandhasamy & Arunachalam, 2008; Silva et al., 2020). Studies show that the antibacterial effect of species belonging to Rhodophyta is more effective (Kolanjinathan & Stella 2009).

In aquaculture units with high fish density, diseases caused by many pathogens are encountered. Fish epidermal mucus is a barrier, providing primary defense against pathogenic microorganisms (Guardiola et al., 2014). The mucus layer contains innate immune components secreted by goblet cells (Spitzer & Koch, 1998; Dash et al., 2018). Mucus composition varies among fish

species and is affected by exogenous and endogenous factors (Jurado et al., 2015). The main compounds of skin mucus are water and glycoproteins containing high molecular weight oligosaccharide molecules called mucins (Guardiola et al., 2014; Jurado et al., 2015). Mucus also form a biochemical barrier containing enzymes such as antimicrobial proteins and proteases contributing to fish immunity (Fast et al., 2002). Immune molecules in mucus include immunoglobulin, lysozyme, lectin, interferon, histones, ribosomal proteins, proteolytic enzymes, antimicrobial peptides, and vitellogenin (Vasta et al., 2011; Ademek et al., 2013; Bergsson et al., 2005). Studies report that fish increase their mucosa or change their composition when exposed to pathogenic bacteria (Van der Marel et al., 2010; Gustafsson, 2013). Many complex molecules, such as lysozymes, phosphatases, esterases, lectins and immunoglobulins in mucus, try to eliminate pathogens and strengthen immunity in case of any infection (Reverter et al., 2018). Many researchers investigated raw fish mucus's antimicrobial properties against infectious pathogens (Hellio et al., 2000; Johansson et al., 2010; Gustafsson et al., 2013). The antimicrobial properties of raw mucus against pathogens were first studied in *Oncorhynchus mykiss* (Rainbow trout) (Austin and McIntosh, 1988).

It is estimated that discards from fishing in the world are approximately 20 million metric tons per year (hellio et al., 2002). In particular, the use of by-products such as mucus and skin obtained from various fish species in biotechnological research has become widespread. Because many bioactive substances can be extracted from fish processing waste (Cunha et al., 2023).

Indiscriminate use of antibiotics in the treatment of infections causes pathogenic bacteria to become resistant to drugs (Kandhasamy & Arunachalam 2008). Therefore, the decreasing effectiveness of antibiotics necessitates the development of new alternatives. In this study, the antimicrobial activities of methanol (M),

acetone (A), ethanol (E), or heptane (H) extracts (E) from *L. papillosa* or *C. officinalis* and the skin and mucus extracts from *S. aurata* on *A. baumannii*, *K. pneumoniae*, *S. aureus*, *E. faecalis*, *C. tropicalis*, and *C. parapsilosis* were investigated.

## 2. Material and Methods

### 2.1. Collection of Samples and Preparation for Experiments

*Laurencia papillosa* (G. Agardh, 1830) and *Carollina officinalis* (Linnaeus, 1758) were collected from Mersin Bay. *S. aurata* (Linnaeus, 1758) were obtained from aquaculture systems. The algae samples were washed with pure water and dried on filter papers. The drained algae samples were dried in the oven and transferred to the grinder. Powdered samples were transferred to falcon tubes and stored in the refrigerator at +4°C. The mucus layer of *S. aurata* was extracted as soon as they were obtained from the cage and transferred to falcon tubes with ice. *S. aurata* were placed in ice and brought to Mersin University, Faculty of Fisheries. They were cleaned from muscle tissue and scales and stored at -18°C.

### 2.2. Preparation of Algae, Fish Mucus, and Skin Extracts

4 g of dry-powdered *L. papillosa* or *C. officinalis* with 40 mL of methanol, acetone, ethanol, or heptane (Merck (Darmstadt, Germany) was mixed with a magnetic stirrer for 24 hours. The samples were filtered using 0.45 filter paper. Aqueous phase and acetic acid extract were prepared from the mucus and skin of *S. aurata* (Uyan, 2020). To obtain aqueous phase extract, 10 mL mucus and 10 mL NaCl (0.85%) were mixed in the tube. The samples were centrifuged at 10000 rpm for 10 minutes to ensure layer formation. After centrifugation, the upper layers of the mucus samples were taken into new tubes. To obtain acetic acid extract, 10g of skin sample and 50 mL of acetic acid (3%) were mixed and homogenized. The homogeneous sample

was kept in hot water for 5 minutes and cooled.

### 2.3. Antimicrobial screening of the extracts

The antimicrobial activity of some extracts of *L. papillosa*, *C. officinalis* and *S. aurata* were investigated using spectrophotometric broth microdilution and disc diffusion methods. Strains used in this study: *Acinobacter baumannii* (ATCC 02026), *Klebsiella pneumoniae* (ATCC 10031), *Staphylococcus aureus* (ATCC 25925), *Enterococcus faecalis* (ATCC 29212) bacterial strains and *C. tropicalis* (ATCC 750) and *C. parapsilosis* yeasts. Before the experiment, bacteria were inoculated on TSA (Tryptic Soy Agar) and yeast were inoculated on SDA (Sabouraud Dextrose Agar) solid medium and incubated at 37°C for 18-24 hours. At the end of 1-day of incubation, the colonies were taken directly from single fallen colonies on the agar plate with a loop and the number of McFarland ( $\sim 10^8$  CFU/mL) was adjusted with physiological saline. Ampicillin was used for bacteria and fluconazole for yeast as positive control antibiotics (Erdoğan Eliuz, 2021).

#### 2.3.1. Determination of MIC (minimum inhibitory concentration) of the extracts

The sterile 96-well plates were prepared for the extracts of *L. papillosa* and *C. officinalis* with methanol, acetone, ethanol and heptane; and the crude, aqueous phase and acetic acid extracts of *S. aurata*. Serial dilutions for each sample were prepared in the horizontal well row of the plate. Starting from the first well of the plate, 50  $\mu$ L of Mueller Hinton Broth (MHB) medium was added to all wells in the microplates. Then, 100  $\mu$ L of the extract was placed in the first well and a double-fold dilution was made until the end of the first ten rows. Positive control, medium control and negative controls were prepared in the last two wells. Finally, 5  $\mu$ L of microorganisms were added

to the wells containing extract and antibiotics and left for incubation. Spectrophotometric measurements (Thermo Scientific, MULTISKAN) were taken at 600 nm and inhibition-concentration graph was drawn. The % inhibition value was obtained using the formula below (Eq. 1). The experiments were repeated 3 times (Erdoğan Eliuz, 2021).

$$\text{Inhibition (\%)} = \left[ 1 - \frac{OD_{\text{test well}}}{OD_{\text{corresponding control well}}} \right] \times 100 \text{ Eq. 1}$$

### 2.3.2. Inhibition zone of the extracts

A certain amount of microorganism solution adjusted according to McFarland 0.5 was spread on the MHA agar petri dish and wells with a diameter of 6 mm were opened in the middle of the petri dish. Each well was filled with 50  $\mu\text{L}$  of the extract and incubated at 37°C for 24 hours. While evaluating the results, the diameters of the inhibition zones were measured in millimeters using the Images program. Sterile distilled water was used as a negative control and all tests were repeated three times (Erdoğan Eliuz, 2021).

### 2.4. Statistical Analysis

IZ and MIC data obtained were statistically evaluated with One Way Anova (post-hoc Tukey HSD Test). Differences ( $p \leq 0.05$ ).

## 3. Results

The antimicrobial activity results of algae, skin, and mucus extracts are comparatively given in Tables 1 and 2. In the ME, where the antimicrobial effects of *L. papillosa* and *C. officinalis* on bacteria and yeasts were investigated, it was determined that the highest inhibition was (5.87 mm) of *C. officinalis* against *E. faecalis*. It was determined that the highest inhibition of *L. papillosa* extracted with acetone was against *K. pneumoniae* (4.77 mm), and the lowest inhibition diameter was against *S. aureus* (2.80 mm). The highest inhibition diameter

of AE of *C. officinalis* was determined as 7.84 mm in *E. faecalis*, and the lowest level was 1.18 mm in *A. baumannii*. *C. officinalis* AE was ineffective against *C. tropicalis*. Both EA of *L. papillosa* and *C. officinalis* more inhibited *E. faecalis* with 6.43 mm and 5.67 mm, respectively, than other microorganisms. The highest inhibition of *L. papillosa* extracted with heptane was calculated as 5.04 mm and 4.59 mm in *C. parapsilosis* and *C. tropicalis* yeasts, respectively. The highest inhibition level in HE of *C. officinalis* was determined as 4.56 mm in *A. baumannii* bacteria. The only bacteria on which the CE prepared from the mucus of *S. aurata* showed an antimicrobial effect was *E. faecalis*, and the inhibition diameter was determined as 1.05 mm. The IZ of the aqueous phase extract prepared from *S. aurata* mucus was determined as 6.14 mm in *E. faecalis*, 7.09 mm in *K. pneumoniae*, and 8.75 mm in inhibition diameter in *C. parapsilosis*. In addition, all pathogens were resistant to acetone, methanol and water used as negative controls.

Interestingly, the aqueous phase extract prepared with *S. aurata* skin had a very high inhibition (13.82 mm) on *C. parapsilosis* (Figure 1).



**Figure 1.** IZ (13.82 mm) of APE of *S. aurata* skin on *C. parapsilosis*.

MICs of *L. papillosa* and *C. officinalis* on gram positive, gram negative and yeast were reported in Table 2. When the MIC levels of *L. papillosa* and *C. officinalis* prepared by methanol were compared, it was determined that *C. officinalis* had a more effective MIC value on bacteria and yeasts.

**Table 1.** Inhibition zone diameters (mm) of algae, mucus and skin extracts against *A. baumannii*, *K. pneumoniae*, *S. aureus*, *E. faecalis*, *C. tropicalis*, *C. parapsilosis*.

	<i>Sa</i>	<i>Ef</i>	<i>Ab</i>	<i>Kp</i>	<i>Cp</i>	<i>Ct</i>
Lp. ME	4.27±1.14 <sup>b</sup>	3.97 ±1.28 <sup>b</sup>	2.63±0.43 <sup>b</sup>	2.65 ±0.04 <sup>b</sup>	1.74 ± 0.00 <sup>b</sup>	4.98±1.49 <sup>b</sup>
C.o. ME	3.96±0.37 <sup>b</sup>	5.87±1.49 <sup>b</sup>	4.07±0.01 <sup>b</sup>	3.43±2.10 <sup>b</sup>	4.05±1.29 <sup>b</sup>	4.11±1.90 <sup>b</sup>
L.p. AE	2.80±0.51 <sup>b</sup>	3.98±0.01 <sup>b</sup>	3.32 ±0.23 <sup>b</sup>	4.77 ±0.57 <sup>b</sup>	3.87 ± 0.41 <sup>b</sup>	3.59±0.73 <sup>b</sup>
C.o. AE	3.83±1.05 <sup>b</sup>	7.84 ± 0.02 <sup>b</sup>	1.18 ± 0.00 <sup>b</sup>	3.78 ±0.00 <sup>b</sup>	1.52 ± 0.02 <sup>b</sup>	-
L.p. EE	5.01±1.79 <sup>b</sup>	6.43 ± 0.08 <sup>b</sup>	3.65 ± 0.04 <sup>b</sup>	4.61±0.75 <sup>b</sup>	2.72 ± 0.00 <sup>b</sup>	2.81±1.25 <sup>b</sup>
C.p. EE	3.76±1.49 <sup>b</sup>	5.67 ± 2.30 <sup>b</sup>	5.54 ± 0.03 <sup>b</sup>	3.08±0.01 <sup>b</sup>	4.14 ± 0.36 <sup>b</sup>	3.23±1.21 <sup>b</sup>
L.p. HE	2.84±0.01 <sup>b</sup>	-	3.05 ± 0.23 <sup>b</sup>	2.07 ± 0.00 <sup>b</sup>	5.04 ± 0.93 <sup>b</sup>	4.59±0.00 <sup>b</sup>
C.o. HE	-	1.50±0.00 <sup>b</sup>	4.56 ± 0.04 <sup>b</sup>	1.84 ± 0.03 <sup>b</sup>	3.79 ± 0.47 <sup>b</sup>	-
S.a. M-CE	-	1.05±0.01 <sup>b</sup>	-	-	-	-
S.a.M-PE	2.76±0.01 <sup>b</sup>	6.14±0.54 <sup>b</sup>	1.38 ± 0.01 <sup>b</sup>	7.09 ± 0.49 <sup>b</sup>	8.75 ± 0.55 <sup>b</sup>	-
S. a. S-CE	-	-	-	-	-	-
S. a. S-APE	-	-	-	-	13.82±0.01 <sup>a</sup>	-
Ant.	32.7±0.01	17.8±0.01	15±0.02	21.0±0.01	19.8±0.03	35.0±0.01

Sa: *Staphylococcus aureus*, Ef: *Enterococcus faecalis*, Ab: *Acinobacter baumannii*, Kp: *Klebsiella pneumoniae*, Cp: *Candida parapsilosis*, Ct: *Candida tropicalis*. Lp: *Laurencia papillosa*, Cp: *Carollina officinalis*, S.au: *Sparus aurata*; M; mucus, S; skin, CE: Crude Extract, APE: Aqueous Phase extract, Acetone extract: AE, Methanol extract: ME, Ethanol extract: EE. Ant: antibiotic (p≤0.05).

For *L. papillosa* ME was 37.41 mg/mL against *C. parapsilosis*, and the highest value was 735.88 mg/mL against *A. baumannii* bacteria. The highest MIC value of ME of *C. officinalis* was determined as 30.42 mg/mL against *E. faecalis*, and the highest value was determined as 86.63 mg/mL against *C. parapsilosis* (p≤0.05).

The lowest MIC values of *L. papillosa* and *C. officinalis* AE were determined against *A. baumannii* as 25.34 mg/mL and 45.92 mg/mL, respectively. The lowest MIC values of *L. papillosa* and *C. officinalis* EA were determined as 31.81 mg/mL and 31.66 mg/mL against *E. faecalis*. In the HEs, the lowest MIC levels of *L. papillosa* and *C. officinalis* were found to be 54.62 mg/mL and 54.62 mg/mL against *C. tropicalis* (p≤0.05).

The most effective (low) MIC values of *S. aurata* were as follows; 88.25 mg/mL for mucus extract on *S. aureus*, 26.73 mg/mL for mucus aqueous phase extract on *C.*

*parapsilosis*; 26.91 mg/mL for skin crude extract on *C. parapsilosis*; 49.87 mg/mL for skin aqueous phase extract on *E. faecalis* (p≤0.05).

#### 4. Discussion

Red algae contain many bioactive compounds with many pharmacological properties. For this reason, especially in recent years, the number of studies on red algae has increased to reveal antimicrobial components (Kolanjinathan & Stella 2009b). Many species, such as *G. edulis* (Kolanjinathan et al., 2009a), *Actinotrichia fragilis* (Salem et al., 2011); *Gracillaria folifera*, *Hypneme muciformis* (Kandhasamy and Arunachalam 2008); have been found to effectively inhibit Gram-positive and Gram-negative bacteria.

**Table 2.** MICs (mg/mL) of algae, mucus and skin extracts against *A. baumannii*, *K. pneumoniae*, *S. aureus*, *E. faecalis*, *C. tropicalis*, *C. parapsilosis* microorganisms

	<i>Sa</i>	<i>Ef</i>	<i>Ab</i>	<i>Kp</i>	<i>Cp</i>	<i>Ct</i>
Lp. ME	107.99±4.72 <sup>a</sup>	104.55±8.25 <sup>a</sup>	735.88±1.7 <sup>b</sup>	94.104±1.94 <sup>a</sup>	37.41±1.20 <sup>a</sup>	64.59±17.8 <sup>a</sup>
C.o. ME	67.29±1.79 <sup>a</sup>	30.42±2.56 <sup>a</sup>	34.42±1.55 <sup>a</sup>	50.15±1.20 <sup>a</sup>	86.63±4.70 <sup>a</sup>	46.28±7.9 <sup>a</sup>
L.p. AE	78.56±3.61 <sup>a</sup>	515.43±9.73 <sup>b</sup>	25.34±0.5 <sup>a</sup>	59.89±1.59 <sup>a</sup>	93.73±7.04 <sup>a</sup>	96.47±3.42 <sup>a</sup>
C.o. AE	140.14±8.46 <sup>a</sup>	140.76±5.27 <sup>a</sup>	45.92±1.81 <sup>a</sup>	434.85±3.77 <sup>b</sup>	60.99±7.63 <sup>a</sup>	74.94±6.22 <sup>a</sup>
L.p. EE	56.35±2.01 <sup>a</sup>	31.81±0.24 <sup>a</sup>	94,17±5,92 <sup>a</sup>	41.93±7.09 <sup>a</sup>	1938.78±1.23 <sup>b</sup>	35.62±3.58 <sup>a</sup>
C.p. EE	101.22±8.02 <sup>a</sup>	31.66±3.78 <sup>a</sup>	38.79±4.18 <sup>a</sup>	60.90±9.96 <sup>a</sup>	88.05±3.54 <sup>a</sup>	37.40±2.10 <sup>a</sup>
L.p. HE	163.63±8.81 <sup>b</sup>	404.56±2.54 <sup>b</sup>	198.76±2.85 <sup>a</sup>	56.62±7.30 <sup>a</sup>	72.71±4.06 <sup>a</sup>	54.62±1.74 <sup>a</sup>
C.o. HE	214.90±9.50 <sup>b</sup>	45.53±3.44 <sup>a</sup>	607.96±7.91 <sup>a</sup>	133.22±1.05 <sup>a</sup>	125.87±4.46 <sup>a</sup>	54.62±1.74 <sup>a</sup>
S.a. M-CE	88.25±2.91 <sup>a</sup>	130.79±4.72 <sup>a</sup>	134.99±1.04 <sup>a</sup>	101.97±7.93 <sup>a</sup>	246.13±1.17 <sup>a</sup>	378.78±2.97 <sup>a</sup>
S.a.M-PE	86.61±2.69 <sup>a</sup>	240.20±2.07 <sup>a</sup>	93.78±4.41 <sup>a</sup>	76.36±4.64 <sup>a</sup>	26.73±2.86 <sup>a</sup>	756.69±6.99 <sup>b</sup>
S. a. S-CE	78.22±8.72 <sup>a</sup>	91.23±1.70 <sup>a</sup>	122.67±6.86 <sup>a</sup>	110.60±4.14 <sup>a</sup>	26.91±1.50 <sup>a</sup>	152.81±8.72 <sup>a</sup>
S. a. S-APE	50.97±4.25 <sup>a</sup>	49.87±1.30 <sup>a</sup>	143.27±7.17 <sup>a</sup>	311.79±1.27 <sup>b</sup>	246.13±1.17 <sup>a</sup>	67.34±2.44 <sup>a</sup>
Ant. (µg/mL)	68.7±0.01	12.7±0.02	92.1±0.01	88.1±0.01	128±0.03	48.7±0.02

Sa: *Staphylococcus aureus*, Ef: *Enterococcus faecalis*, Ab: *Acinobacter baumannii*, Kp: *Klebsiella pneumoniae*, Cp: *Candida parapsilosis*, Ct: *Candida tropicalis*. Lp: *Laurencia papillosa*, Cp: *Carollina officinalis*, S.au: *Sparus aurata*; M; mucus, S; skin, CE: Crude Extract, APE: Aqueous Phase extract, Acetone extract: AE, Methanol extract: ME, Ethanol extract: EE. Ant: antibiotic (p≤0.05).

A few antimicrobial activity studies with *L. papillosa* extracts have been found in the literature. Among them, Kavita et al., (2014) reported the inhibition zone of *L. papillosa* methanol extract on *E.coli* (Gram -), *S. aureus* (Gram +), *B. subtilis* (G +), *P. aeruginosa* (G -) bacteria as 12.33 mm, 14.33 mm, 11.66 mm, respectively. In our study, the most effective inhibition of *L. papillosa* was against *S. aureus* (G +), and *E. faecalis* (G +), with 6.43 mm and 5.01 mm, respectively. The difference in the level of inhibition may result from technical differences in the extraction step. In another study, the antimicrobial potential of *C. officinalis* on *E. faecalis* (G +), *E. aerogenes* (G -) and *E. coli* (G -) was revealed by Taşkın et al. The inhibition level of *C. officinalis* extract on *E. faecalis* and *E. coli* bacteria was reported as 21.66 mm and 32 mm, respectively. This study supports our finding that *C. officinalis* is effective on *E. faecalis*. The highest inhibition of *Corallina officinalis* on *E. faecalis* (G+) was realized with acetone (7.84 mm) and ethanol (5.67 mm) extracts.

It is known that the antimicrobial potential in macroalgae is generally due to the polyphenolic compounds it contains (Silva et al., 2020). In particular, green and red algae contain many secondary metabolites, including catechin, phlorotannin, phenolic acids and flavonols (Gómez-Guzmán et al., 2018; Cassani et al., 2020). *L. papillosa* has been found to be very rich in substances such as vanillin, hydroxytyrosol, urolithin A, phloroglucinol, 2-Hydroxy-2-phenylacetic acid, quercetin caffeoyl-glucoside, p-Coumaric acid methyl ester-p-Hydroxybenzoic acid, p-Hydroxybenzaldehyde and sinapic acid (Goksen 2023). The presence of p-hydroxybenzaldehyde in red algae has also been shown previously (Rajauria et al., 2016; Nørskov et al., 2021) and it has been determined to be an important antioxidant (Zhong et al., 2020) and antimicrobial (Taib et al., 2020). Similarly, phenolic acids such as salicylic acid, p-hydroxybenzoic acid, gentisic acid, protocatechuic acid, gallic acid, vanillic acid, and syringic acid were found

abundantly in *Gracilaria species* (Xu et al., 2015; Dhaouafi et al., 2023).

When the antimicrobial effects of algae were compared with *S. aurata* mucus, similar results were observed with the phenolic contents of macroalgae. The antimicrobial effect of *S. aurata* mucus extract on *E. faecalis* was found to be almost the same as *L. papillosa* (6.14 mm and 6.43 mm). The inhibitions of *S. aurata* aqueous phase extract on *K. pneumoniae* and *C. parapsilosis* (7.09 mm) were much greater than the effects of *L. papillosa* and *C. officinalis* extraction. This means that *S. aurata* mucus content is more effective on *K. pneumoniae* and *C. parapsilosis* than the phenolic content of both algae. It has been previously reported that mucus may have antimicrobial effects due to its structure and chemical content. A study reported that cupra skin mucus contained lower levels of lysozyme, alkaline phosphatase and protease, and higher esterase, peroxidase and antiprotease activities. (Guardiola et al., 2014). Additionally, that heavy metals in sea environment were affected the enzymatic changes and bactericidal activity in the mucus layer in *S. aurata* (Guardiola et al., 2015). Cordero et al. (2016) reported that a decrease was observed in the level of total sugar and protein residues, in protease, peroxidase and lysozyme activities compared to fresh samples during fresh and freezing of mucus samples in *S. aurata*. In another study, bioactive metabolites of *P. sophore* mucus extract were analyzed by HR-LCMS and reported that the mucus content consisted of compounds such as cysteamine, glucosamine, phytosphingosine, arachidonoyl amine, 2-amino-tetradecanoic acid, 2,4-dimethyl-tetradecanoic acid, dihydrosphingosine (Reid et al., 2020). In a study, Subramanian et al. (2008) conducted that high inhibition was reported on the *Salmonella enterica* strain by extracting (acidic, organic and aqueous solvents) mucus samples of various fish species (*Salvelinus alpinus*, *S. fontinalis*, *Cyprinus carpio*, *Melanogrammus aeglefinus*). In the same study, they did not detect any antimicrobial

effect in aqueous mucus extracts (Subramanian et al., 2008). In another study, Hellio et al. (2002) reported that extracts obtained from fish epidermis and epidermal mucus did not inhibited gram-negative and gram-positive bacteria. However, they detected antibacterial effects in ethanolic and dichloromethane fractions. It is understood that the antimicrobial effects of extracts prepared with fish mucus and skin samples may vary depending on the climate, type of fish, and technical conditions.

## 5. Conclusion

As a result, the antimicrobial activities of *L. papillosa* and *C. officinalis* red algae, which are rich in phenolic content, showed average activity on the microorganisms we studied. It is understood that *S. aurata* mucus and skin extracts are very effective, especially on *K. pneumoniae*, *A. baumannii* and *Candida* sp., which were studied for the first time. When compared to the phenolic richness in macroalgae, it is understood that the enzymes and protein structures that can be found in the mucus and skin of *S. aurata* were almost as effective as phenols. In future studies, extracts of both organisms can be studied by creating complex structures together in order to enrich each other with bioactive compounds.

## Compliance with Ethical Standards

### Conflict of interest

The authors declare that they have no competing interests.

### Author contribution

All authors' contributions are equal for the preparation of research in the manuscript. All the authors verify that the Text, Figures, and Tables are original and that they have not been published before.

### Ethical approval

Ethics committee approval is not required.

### Acknowledgments

This study was supported by the Research Fund of Mersin University in Türkiye with Project Number: 2020-1-TP3-4042.

### Data availability

Not applicable.

### Consent for publication

Not applicable.

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