

## Original Research Article

# Antimicrobial properties of bioactive compounds isolated from epidermal mucus in two Ray species (*Dasyatis marmorata* and *Gymnura altavela*)

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Sent for review: 13 March 2020

Revised accepted: 21 September 2020

### Abstract

**Purpose:** To investigate the occurrence and antimicrobial effects of certain biochemical compounds in the epidermal mucus secretions of fish and to demonstrate their potential for biomedical applications.

**Methods:** Crude, aqueous, and acidic epidermal mucus samples were collected from live ray specimens. Gas chromatography and gas chromatography-mass spectrometry (GC/MS) analyses were performed to identify the biochemical compounds present in the mucus. The spectrophotometric broth microdilution method was used to determine the antibacterial and antifungal properties of the mucus extracts. The bacterial strains, *Bacillus subtilis*, *Escherichia coli*, *Enterococcus faecalis*, and *Klebsiella pneumoniae*, were used for the tests, as well as the fungal strains, *Candida parapsilosis* and *Candida albicans*.

**Results:** GC/MS analysis revealed the presence of several hydrocarbon-derived compounds in the epidermal mucus of the two ray species. The acidic extract of *G. altavela* epidermal mucus produced a high MIC value, indicating the highest inhibitory effect of 8.64  $\mu$ L against *E. coli*, while the crude extract of *G. altavela* epidermal mucus (41.13  $\mu$ L against *B. subtilis*) was the least effective.

**Conclusion:** Epidermal mucus extracts, especially when acid-based, displays strong antimicrobial properties against all the tested pathogens. These findings suggest the plants possess some potential for the development of novel antimicrobial components for applications in medicine.

**Keywords:** Fish, Ray species, Epidermal mucus, Antimicrobial properties, Bioactive compounds

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## INTRODUCTION

In fishes, the external body surface generally is covered by a mucus coat secreted by epidermal gland cells. The mucus layer differs significantly in density and viscosity among fish species, and

it is essential for fishes in several respects. Goblet cells are the major components of the mucus layer. While all mucosal surfaces in fishes have multiple distinct and complicated mechanisms for defense against pathogenic diseases, the skin mucus functions as the

primary barrier against pathogens and environmental stressors [1].

Mucus is the slimy and sticky secretion that consists of mucins and a mixture of other materials, such as inorganic salts, immunoglobulins, and water-suspended lipids that give it distinctive lubricating characteristics. The mucins can be described as viscous colloids comprised of bacteria, proteins, and water. Mucins contain a variety of immune-relevant compounds that are defined as inherent defense variables, including glycoproteins, glycosaminoglycans, pheromones, proteolytic enzymes, lectins, galectins, lysozymes, calmodulins, immunoglobulins, complements, C-reactive proteins and antimicrobial peptides (AMP). Numerous other antimicrobial peptides also provide defense mechanisms for fish against possible pathogens and potential environmental risks [1,2].

A few studies have been carried out on the antimicrobial properties of fish epidermal mucus in ray species that include the common stingray (*Dasyatis pastinaca*) [3,4], sharpnose stingray (*Himantura gerrardi*) [4], and bigtooth river stingray (*Potamotrygon cf. henlei*) [5]. The three aforementioned studies also investigated the antimicrobial effects of epidermal mucus (acidic, crude, and aqueous extracts) of ray species against for both gram-positive and gram-negative bacterial strains, as well as against fungal strains.

Information about the antimicrobial properties and biochemical structures of fish epidermal mucus may help in developing antimicrobial drugs for therapeutic purposes. To the best of our knowledge, there have been no studies on antimicrobial properties of epidermal mucus obtained from the marbled stingray *Dasyatis marmorata* or the spiny butterfly ray *Gymnura altavela*. Also, there is an insufficient number of studies about GC/MS analysis of fish mucus in the literature. Consequently, the present study aimed to isolate biological compounds of the epidermal mucus of *Dasyatis marmorata* and *Gymnura altavela* and to determine the antimicrobial effects of their epidermal mucus extracts.

## EXPERIMENTAL

### Collection of fish epidermal mucus samples

Epidermal mucus was separated from the dorsal body surface of the fish with a sterile plastic spatula. Mucus was not collected from the ventral sides of the fish to avoid contamination by

intestinal and urinogenital excretions [6]. A pooled sample of 50 mL of mucus was collected from each ray species and put into a sterile Falcon tube per species. The samples were stored in a bag filled with ice for transport to the laboratory and then stored at  $-30\text{ }^{\circ}\text{C}$  in the laboratory pending further analysis.

### Preparation of fish epidermal mucus extracts

Crude extracts of epidermal mucus were prepared according to the method described by Mat Jais *et al* [7]. Initially, 10 mL of mucus was transferred to ten Eppendorf tubes in equivalent quantities and centrifuged at 5000 rpm for 10 minutes. Then the obtained supernatant phase was stored at  $4\text{ }^{\circ}\text{C}$  in a refrigerator for antimicrobial assays.

The aqueous extract of epidermal mucus was prepared according to the process of Tyor & Kumari [8]. Initially, 10 mL of mucus was transferred to each of ten Eppendorf tubes in equivalent quantities and was mixed with an equal amount of sterilized physiological saline solution (0.85% NaCl) and centrifuged at 5000 rpm for 5 minutes. Then the supernatant phase was kept at  $4\text{ }^{\circ}\text{C}$  in a refrigerator for antimicrobial assays.

The acidic extract of epidermal mucus was prepared as described by Subramanian *et al* [2]. Initially, 30 mL of fish mucus was added to 30 mL of 3% acetic acid and heated for 5 minutes in boiling water. Then, the acid-mucus mixture was then cooled in the icebox and homogenized. The mixture was centrifuged at 18000 g and  $4\text{ }^{\circ}\text{C}$  for 35 minutes. Then the resultant supernatant liquid was purified through a 0.22- $\mu\text{m}$  filter syringe. Finally, the obtained elutes were stored in a refrigerator at  $4\text{ }^{\circ}\text{C}$  for antimicrobial assays.

The hexane extraction method was chosen to separate the volatile compounds from the epidermal mucus for GC/MS analysis using the modified protocol of Christy-Shaila *et al* [9]. The mucus sample (2 mL) and hexane (2 mL) were added to the sample tube and extracted with an ultrasonic bath (1 min.) and vortex (1 min.). The hexane phase was separated from the water after standing 30 minutes under laboratory conditions. The hexane extract was filtered with a 0.45- $\mu\text{m}$  syringe filter to prepare it for GC/MS analysis.

### Bradford assay

Total protein concentration in the crude extracts of epidermal mucus was determined based on the method introduced by Bradford [10] with

bovine serum albumin (BSA) as the standard (Bio-Rad). Briefly, 1 mL of epidermal mucus sample was mixed with 1 mL of Bradford reagent, incubated at ambient temperature for 10 min and read at 595 nm with a Thermo Evolution 160 UV-VIS spectrophotometer. A standard curve ranging from 0–2.0 µg/mL was generated with absorbance at 595 nm, and the protein content of the epidermal mucus was calculated for each ray species.

### GC-MS analysis

A mucus sample of 1.0 µL was injected splitlessly using a Finnigan-Trace GC/MS unit with an autosampler. The capillary column used was Zebron ZB-5 (5% phenyl-95% dimethylpolysiloxane), 0.25 mm i.d. x 60 m, film thickness 0.25 µm. Helium was used as the carrier gas at a stable flow rate of 1.0 mL/min. The inlet temperature was set at 260 °C. The column temperature was first kept at 70 °C for 1 minute, then increased to 260 °C at 6 °C/min, and finally increased to 300 °C at 25 °C/min, where it was kept for 5 minutes. The column effluent was introduced into the MS ion source at 230 °C.

The mass spectrometer was run in the electron impact (EI) mode at 70 eV. Data was acquired in the full scan mode from 50 to 550 m/z, with the scan time being 0.5 sec. Data analysis was performed using total ion count (TIC) for compound recognition and quantification. The components' spectra and the database of spectra of the known components kept in the GC/MS library were compared. Quantitative determination was performed on the basis of peak area integration.

### Preparation of microbial strains

The antimicrobial effects of acidic, crude, and aqueous extracts of the two ray species were investigated by the spectrophotometric broth microdilution method. Four bacterial strains—two gram-negative bacteria, *Escherichia coli* (ATCC 25293) and *Klebsiella pneumoniae*, and two gram-positive bacteria, *Bacillus subtilis* (ATCC 6633) and *Enterococcus faecalis*—as well as two fungal strains, *Candida albicans* and *C. parapsilosis*, were used to investigate antimicrobial activities of the mucus extracts. Test microorganisms were inoculated with 4 mL of Tryptic Soy Broth for the bacteria and 4 mL of Sabouraud Dextrose Broth for the yeasts. Both batches were incubated at 37 °C for 24 hours. According to 0.5 McFarland standard turbidity ( $\approx 10^4$  for bacteria and  $\approx 10^3$  for yeasts), the microorganism suspensions were prepared after

one day of incubation and were kept at +4 °C for use in spectrophotometric broth microdilution experiments. Ampicillin and Fluconazole were used as positive controls for bacteria and yeasts, respectively.

### Screening of fish epidermal mucus extracts for antimicrobial properties

The method of microbroth dilution was used to determine the minimum inhibitory concentration (MIC), the lowest concentration of analyzed substances that prevents the growth of 99.9% of microorganisms, in according with the guideline of the U.S. National Committee for Clinical Laboratory Standards. After a 50 µL portion of Mueller Hinton Broth (MHB) solution had been poured into each of the first-order wells of a 96-well microplate, a 100-µL portion of extract was added to each well. Then twofold serial dilutions of extracts were made to produce a final diluted sample concentration of 0.19 µL. Finally, 5 µL of each microorganism was added into the wells. The same procedure was performed for the antibiotics. After all 96-plates had been incubated for 24 hours at 37 °C, spectrophotometric analysis was performed at 600 nm for bacteria and 415 nm for yeasts to measure turbidity.

### Data analysis

The MIC was calculated by using Microsoft Excel 2013 computer software. The experiment was repeated at least three times, and the SPSS one-way ANOVA Tukey test was performed separately for MICs. The results were recorded as mean  $\pm$  SEM (standard error of the mean).

## RESULTS

### Protein concentration

On the basis of the seven-point standard calibration curve, ranging from 0–2 mg/mL ( $y = 0.5158x + 0.0845$ ;  $R^2 = 0.9554$ ), the total protein concentration of epidermal mucus was  $4.43 \pm 0.08$  mg/mL for *D. marmorata* and  $4.75 \pm 0.08$  mg/mL for *G. altavela*.

### Bioactive compounds

Gas chromatography-mass spectrometry analysis, using hexane solution, was carried out on the epidermal mucus of two ray species. In the biochemical analysis of *D. marmorata* and *G. altavela* epidermal mucus, sixteen and fourteen compounds were identified, respectively. The major bioactive compounds in the mucus of *D. marmorata* were identified as 2-Isopropyl-5-methyl-1-heptanol (10.04%), 2-tert-Butyl-4-

isopropyl-5-methylphenol (7.95%), docosane (7.98%), and dodecanoic acid (15.76%). For the mucus of *G. altavela*, they were 1-nonene (8.13%), 2-Isopropyl-5-methyl-1-heptanol (12.24%), 2-Hydroxy-3-phenyl-2H-1-benzopyran (11.83%), eicosane (14.25%), and dodecanoic acid (21.37%). The chromatograms of the GC/MS profile for each mucus are given in Figures 1 and 2. The peaks in the chromatogram were incorporated and then compared with the database spectra of the known components in the GC/MS library. Tables 1 and 2 show the results of GC/MS analysis of epidermal mucus.

The MIC values of acidic (DmAC), crude (DmCR) and aqueous (DmAQ) mucus extracts of *D. marmorata*, and acidic (GaAC), crude (GaCR) and aqueous (GaAQ) mucus extracts of *G. altavela* are summarized in Table 3. The values,

after incubation with microorganisms for 24 h, ranged from 8.64 to 41.13  $\mu$ L for the tested pathogens ( $n = 18$ ) ( $P < 0.05$ ). In general, all extracts below 50  $\mu$ L showed antimicrobial properties. The most effective MIC value was 8.64  $\mu$ L for GaAC extract against *E. coli*, followed by those of DmAC extract against *K. pneumoniae* (8.99  $\mu$ L), GaAC extract against *K. pneumoniae* (9.01  $\mu$ L), DmAC extract against *E. coli* (9.30  $\mu$ L), and GaAC extract against *B. subtilis* (9.40  $\mu$ L).

The antimicrobial activity of the DmAQ extracts against *C. parapsilosis* (MIC = 38.05  $\mu$ L) was lower and statistically different from those of other extracts (DmAC, DmCR, GaAC, GaCR, and GaAQ) ( $P < 0.05$ ), as shown in Table 3.

**Table 1:** Chemical compounds of *D. marmorata* epidermal mucus identified via GC/MS

No.	Retention time	Peak area (%)	Molecular weight	Molecular formula	Name
1	16.52	7.45	200	C <sub>13</sub> H <sub>28</sub> O	Isotridecanol
2	16.71	10.04	172	C <sub>11</sub> H <sub>24</sub> O	2-Isopropyl-5-methyl-1-heptanol
3	16.89	4.58	140	C <sub>10</sub> H <sub>20</sub>	1,2,3,5-tetramethylcyclohexane
4	19.88	2.00	198	C <sub>14</sub> H <sub>30</sub>	Dodecane
5	20.55	3.17	198	C <sub>14</sub> H <sub>30</sub>	Tridecane
6	21.04	7.95	206	C <sub>14</sub> H <sub>22</sub> O	2- <i>tert</i> -Butyl-4-isopropyl-5-methylphenol
7	21.47	7.98	310	C <sub>22</sub> H <sub>46</sub>	Docosane
8	21.70	7.47	214	C <sub>14</sub> H <sub>30</sub> O	2-Hexyl-1-octanol
9	21.90	5.79	186	C <sub>12</sub> H <sub>26</sub> O	1-Octanol
10	23.26	15.76	242	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	Dodecanoic acid
11	24.69	4.25	366	C <sub>26</sub> H <sub>54</sub>	Octadecane
12	25.24	3.29	242	C <sub>16</sub> H <sub>34</sub> O	1-Decanol
13	25.48	4.88	380	C <sub>27</sub> H <sub>56</sub>	Heptacosane
14	25.75	5.98	266	C <sub>19</sub> H <sub>38</sub>	1-Nonadecene
15	26.13	5.79	294	C <sub>21</sub> H <sub>42</sub>	10-Heneicosene
16	26.33	3.60	490	C <sub>35</sub> H <sub>70</sub>	17-Pentatriacontene

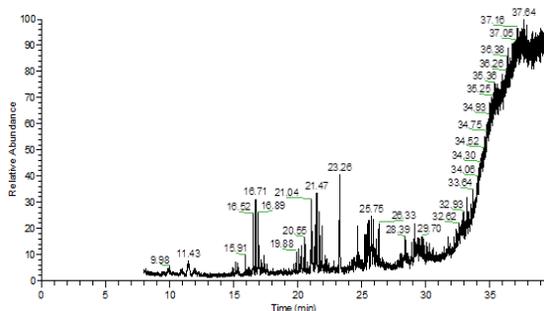
**Table 2:** Bioactive compounds of *G. altavela* epidermal mucus identified via GC/MS

No.	RT	Peak Area (%)	Molecular weight	Molecular formula	Name
1	15.13	2.24	184	C <sub>13</sub> H <sub>28</sub>	Undecane
2	15.31	2.38	212	C <sub>15</sub> H <sub>32</sub>	Pentadecane
3	16.53	8.13	168	C <sub>22</sub> H <sub>46</sub>	1-Nonene
4	16.72	12.24	172	C <sub>11</sub> H <sub>24</sub> O	2-Isopropyl-5-methyl-1-heptanol
5	21.05	11.83	224	C <sub>15</sub> H <sub>12</sub> O <sub>2</sub>	2-Hydroxy-3-phenyl-2H-1-benzopyran
6	21.17	4.63	214	C <sub>14</sub> H <sub>30</sub> O	2-Hexyl-1-octanol
7	21.72	14.25	282	C <sub>20</sub> H <sub>42</sub>	Eicosane
8	23.27	21.37	242	C <sub>15</sub> H <sub>30</sub> O <sub>2</sub>	Dodecanoic acid
9	25.26	4.18	258	C <sub>16</sub> H <sub>34</sub> S	<i>tert</i> -Hexadecanethiol
10	26.35	4.65	172	C <sub>11</sub> H <sub>24</sub> O	2-Isopropyl-5-methyl-1-heptanol
11	29.87	3.25	366	C <sub>26</sub> H <sub>54</sub>	Octadecane
12	30.10	3.58	380	C <sub>27</sub> H <sub>56</sub>	Heptacosane
13	33.24	3.98	314	C <sub>20</sub> H <sub>42</sub> O <sub>2</sub>	2-Octadecyloxyethanol
14	36.43	3.29	578	C <sub>16</sub> H <sub>50</sub> O <sub>7</sub> Si <sub>8</sub>	Octasiloxane

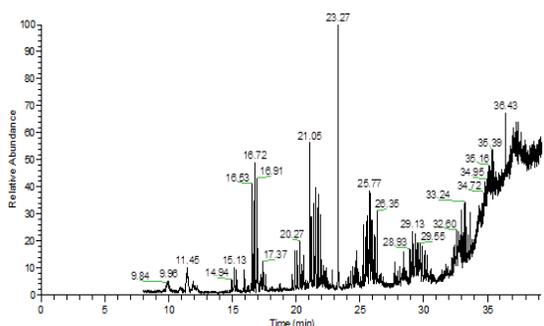
**Table 3:** MIC values of epidermal mucus extracts, determined by the spectrophotometric microdilution method

Extract (µl)	MIC + SEM*					
	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>B. subtilis</i>	<i>E. faecalis</i>	<i>C. albicans</i>	<i>C. parapsilosis</i>
DmAC	9.30 <sup>a</sup> ±0.08	8.99 <sup>b</sup> ±0.5	10.23 <sup>b</sup> ±0.1	9.19 <sup>b</sup> ±0.06	9.57 <sup>a</sup> ±0.2	9.77 <sup>b</sup> ±0.3
DmCR	12.78 <sup>a</sup> ±2.9	14.22 <sup>b</sup> ±5.6	14.69 <sup>b</sup> ±3.5	24.17 <sup>b</sup> ±9.1	15.53 <sup>a</sup> ±2.3	9.81 <sup>b</sup> ±0.1
DmAQ	37.03 <sup>a</sup> ±24.2	19.02 <sup>b</sup> ±3.7	22.15 <sup>b</sup> ±6.6	16.54 <sup>cb</sup> ±5.2	20.60 <sup>a</sup> ±4.06	38.05 <sup>a</sup> ±15.2
GaAC	8.64 <sup>a</sup> ±0.1	9.01 <sup>b</sup> ±0.1	9.40 <sup>b</sup> ±0.1	9.13 <sup>b</sup> ±0.2	9.40 <sup>a</sup> ±0.01	9.44 <sup>b</sup> ±0.1
GaCR	18.97 <sup>a</sup> ±2.5	27.84 <sup>ab</sup> ±3.3	41.13 <sup>ab</sup> ±8.9	23.26 <sup>b</sup> ±6.0	21.99 <sup>a</sup> ±17.8	16.92 <sup>b</sup> ±7.6
GaAQ	22.58 <sup>a</sup> ± 0.02	14.88 <sup>b</sup> ±4.1	16.97 <sup>b</sup> ±6.3	16.16 <sup>b</sup> ±2.5	10.86 <sup>a</sup> ±0.9	21.39 <sup>ab</sup> ±7.6
Ampicillin (µg/mL)	16 ±0.06	8.99 <sup>b</sup> ±0.5	32 ±0.4	32 ±0.3	-	-
Fluconazole (µg/mL)	-	-	-	-	128 ±3.5	128 ±0.5

\*SEM: Standard error of the mean. Mean MICs are expressed with the standard deviation (±) and significance level (ANOVA, *P* < 0.05, Tukey's test). Values on the same column with different superscript letters differ statistically from the 0.05 probability



**Figure 1:** GC/MS chromatogram of *D. marmorata* epidermal mucus



**Figure 2:** GC/MS chromatogram of *G. altavela* epidermal mucus

**DISCUSSION**

This study examines the total protein concentrations, bioactive compounds, and antimicrobial activities of the epidermal mucus secretions of *Dasyatis marmorata* and *Gymnura altavela*. Previous studies have suggested that inhibitory functions and biochemical structures of the mucus of various fishes may change on the basis of various pathogens, inter- and intra-fish species diversity, ecological and physiological conditions, nutritional impact, environmental

stresses such as changes in water pH or salinity, and the growth and maturity of the fishes. Even differences in laboratory protocols or conditions of sample storage may affect those characteristics [2,11,12].

Total protein analysis showed a higher protein concentration for the analyzed epidermal mucus of the two ray species when compared to several studies on other fish species [2,13-15]. The protein content in the epidermal mucus may vary based on various stress factors of fish—environmental factors (e.g. water quality, i.e. the values of DO, CO<sub>2</sub>, ammonia, and pH), presence of contaminants, and differences between fish species. Subramanian *et al* [2] found that the mucus-producing cells in the epidermal and epithelial layers show difference among fish species, and therefore, they may affect the composition of mucus.

GC/MS analysis revealed that the hexane extracts of the epidermal mucus from each ray species contained numerous volatile compounds. Among the identified major compounds in the epidermal mucus of *D. marmorata*, antimicrobial activities were reported for the bioactive compound 2-isopropyl-5-methyl-1-heptanol [16] and the alkane docosane [17]. Antibacterial, antifungal, and antiviral properties have been observed in the saturated fatty acid dodecanoic acid [18]. The biological activity of 2-*tert*-Butyl-4-isopropyl-5-methylphenol has not been clearly identified [19]. In the epidermal mucus of *G. altavela*, antibacterial and antifungal properties were found in the alkane 1-nonene [20], and anticancer and antimicrobial effects were reported for the alkane eicosane [21]. Similarly, 2-isopropyl-5-methyl-1-heptanol and dodecanoic acid were also observed as important bioactive compounds. No biological properties have been reported for 2-hydroxy-3-phenyl-2H-1-

benzopyran to date. Christy-Shaila *et al* [9] analyzed biochemical components in the mucus of zebrafish (*Danio rerio*), and, similar to the results of this study, they found eighteen compounds, all of which were hydrocarbons.

Antimicrobial activity against all the test pathogens was also detected in the aqueous and crude extracts. The MIC results are at variance with previous studies on the aqueous mucus extracts of *Pollachius virens*, *Gadus morhua*, *Labrus bergylta*, *Scophthalmus rhombus*, *Platichthys flesus* and *Solea solea* against *B. subtilis*, *E. coli*, *K. pneumoniae*, and *C. albicans* [6], and with those of the crude and aqueous mucus extracts of *Channa striatus* against *B. subtilis* and *K. pneumoniae* [13]. The discrepancies in antimicrobial effects of the same types of epidermal mucus extracts obtained from various fish species may originate from structural differences in their excreted mucus.

As shown by the MIC values of the acidic mucus extracts in this study, strong antimicrobial effects were obtained against all the used pathogens in the range of 8.99–10.23 µg/mL for DmAC and 8.64–9.44 µg/mL for GaAC. Those values show greater inhibitory effectiveness than was found in previous studies on acidic mucus extracts carried out on *Oreochromis niloticus* and *Mystus nemurus* against *E. coli*, *B. subtilis* and *K. pneumoniae* [14], *Salvelinus fontinalis* and *Melanogrammus aeglefinus* against *E. coli* [2], and *Dasyatis pastinaca* and *Himantura gerrardi* against *K. pneumoniae*, *E. coli*, and *C. albicans* [4]. They showed less effectiveness than did the studies of *Myxine glutinosa* mucus against *E. coli* [2], *Potamotrygon cf. henlei* against *E. coli* [5], and *Channa striatus* against *B. subtilis* and *K. pneumoniae* [13]. Therefore, specific acidic extracts that can be used as antimicrobial compounds need to be characterized as biomaterials for further biomedical examinations, especially against common pathogens.

This study includes preliminary results that may contribute to research in the use of biomaterials from the marine environment for biomedical applications. It is known that antibiotic resistance is among the most serious problems in medicine. Bacteria are among organisms that can reproduce and mutate very quickly. For that reason, prescribed antibiotic drugs tend to lose their effectiveness against certain bacterial species over time, and that causes problems in the treatment of some bacterial diseases [22]. Although increasing the dose increment of antibiotics may be evaluated as an option, the possible side effects have prompted researchers to look for biomaterial alternatives. Likewise, it is

known that antifungal drugs are relatively unsafe and that effective antifungal drugs have yet to be developed due to the cellular similarity of fungi and mammals [23]. The azole group of antifungal agents, Fluconazole, Myconazole, Itraconazole, and Ketoconazole, has been used clinically. Because the side effects of those drugs, nephrotoxicity and hepatotoxicity, can include severe gastric distress [24], there remains a strong demand for the development of reliable antifungal drugs.

## CONCLUSION

The most potent antimicrobial properties found in this study are exerted by the acidic mucus extracts of the two ray species. This shows that acid-soluble proteins may be key defense components against common pathogens. Further characterization of the antimicrobial substances may also give insight into possible human health-related applications as biomaterials, including uses such as antibacterial, antifungal, antiviral, anticancer, anti-hepatotoxic, antispasmodic, anti-rheumatic and antagonistic agents, as well as applications such as bulking agents, release media, protective coatings, lubricants, and emulsifiers.

## DECLARATIONS

### Acknowledgement

This study was derived from A Uyan's PhD Thesis. Thanks go to the Scientific and Technological Research Council of Turkey (no. TUBITAK-2211/C National Ph.D. Scholarship Program for Priority Areas) for support.

### Conflict of interest

No conflict of interest is associated with this work.

### Contribution of authors

We declare that this work was done by the authors named in this article and that all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Ali Uyan and Cemal Turan performed the bibliographic research and drafted the manuscript. Elif Ayse Erdogan-Eliuz, and Ali Uyan analyzed the antimicrobial properties of fish mucus. Kemal Sangun, Ali Uyan, and Cemal Turan carried out GC/MS analysis. Cemal Turan and Ali Uyan contributed to all the writing and correction of the manuscript. All the authors reviewed the final

version of the manuscript and contributed to the discussion of the data.

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