WALAILAK JOURNAL
http://wjst.wu.ac.th Health Sciences

Antimicrobial Activity of Epidermal Mucus from Top Aquaculture Fish Species against Medically-Important Pathogens[†]

Gary Antonio Ceñidoza LIRIO[1,*], Jinky Alyssa Aquino DE LEON[2] and Airha Garcia VILLAFUERTE[2]

[1]*Institute for Science and Technology Research, Center for Life Sciences Research, Polytechnic University of the Philippines, Metro Manila, Philippines*
[2]*College of Science, Department of Biology, Polytechnic University of the Philippines, Metro Manila, Philippines*

([*]Corresponding author's e-mail: garylirio@gmail.com)

Received: 5 March 2018, Revised: 25 July 2018, Accepted: 15 August 2018

Abstract

Zoonotic infections caused by bacterial pathogens are considered as major threat to humans and the aquaculture industry. This problem triggered the search for various natural products from plants, microorganisms, animal tissues, and secretions to determine the presence of metabolites that may be of potential antimicrobial effects against infectious agents. However, limited attempts have been conducted to elucidate the potential use of freshwater fish mucus in against pathogens. Here, the antimicrobial activity of mucus of economically-important freshwater fish species in the Philippines: *Oreochromis niloticus* (tilapia), *Clarias batrachus* (catfish), and *Channa striata* (snakehead fish) was investigated against fish and human pathogens. The pooled fish mucus was extracted with succeeding centrifugation and filtration. The acidic mucus extracts were tested for antimicrobial-inhibitory effects and minimum inhibitory concentration (MIC) by agar-overlay diffusion and microbroth plate dilution method, respectively. The results showed that all fish mucus extracts exhibited antimicrobial effects against test pathogens with catfish exhibiting the highest inhibitory effects against *Pseudomonas aeruginosa* (p = 0.096), *Klebsiella pneumoniae* (p = 0.000), *Enterococcus faecalis* (p = 0.665), *Micrococcus luteus* (p = 0.000), *Aeromonas hydrophila* (p = 0.000), *Staphylococcus aureus* (p = 0.000), *Escherichia coli* (p = 0.000), and *Serratia marcescens* (p = 0.000) as compared to the broad-spectrum antibiotic control, Cefoperazone. Interestingly, catfish mucus revealed inhibitory effects against Gram-positive *S. aureus* and *M. luteus* at the lowest concentration (1:4 dilution). The present findings revealed the potential antimicrobial use of freshwater fish mucus against medically-important pathogens.

Keywords: Fish mucus, freshwater fish, antimicrobial, agar-well diffusion, minimum-inhibitory concentration

Introduction

Outbreaks of zoonotic diseases have increased in the past few decades causing massive socioeconomic impact worldwide [1]. Zoonosis is the direct transmission of diseases from animals to humans through various routes. Causative agents for the disease include several organisms, where most of the cases documented were caused by bacteria [2], infecting wide-range of hosts including fish species and humans. Several bacterial pathogens have been known to cause infectious diseases resulting in

[†]Presented at the International Conference on Biomedical Sciences 2018: March 22nd - 23rd, 2018

bacterial hemorrhagic ascites, columnaris disease, and Edwardsiellosis in fish [3-5]. Production of fish worldwide for human consumption has been steadily increasing, however, resulted to increased cases of zoonotic diseases linked to transmission of bacterial pathogens from infected fish produce to humans, posing severe threats to human health and aquaculture industry [6]. In the Philippines, aquaculture has been the center of the industry, with most of the fish produced are consumed locally [7], thus the proliferation of bacterial infections among fishes may be acquired unknowingly by humans via ingestion.

Currently, the Philippines is witnessing outbreaks of emerging bacterial infections such as typhoid, salmonellosis, and pneumonia [8]. Infections by bacterial pathogens resulted in the increased number of hospitalized citizens who become unfortunate victims of nosocomial infections contracted from health care facilities and thru invasive medical equipment or surgical procedures [9]. The consequence of the infections leads to prolonged antibiotic usage, hospital costs, and economic losses. Several organisms may cause hospital-acquired infections, where most of the cases were caused by common and drug-resistant bacterial pathogens [10,11].

The pervasiveness of drug resistance has become a threat to the efficacy of antibiotics used to treat infections [12-14]. High dosage of antibiotics acts faster on bacterial infections, hence, increasing the risk for hepatoxicity, neurotoxicity, and other adverse side-effects to humans [15-16]. As a solution, the discovery and development of new alternatives for commercially-available antibiotics are currently being promoted globally. Novel drug alternatives from compounds derived from plants, animals, and microorganisms are considered promising candidates. Natural products are substances produced by organisms found in nature [17]. As compared to commercially-available antibiotics, natural products are believed to be reasonable, environment-friendly, cost-efficient, and have less adverse side-effects [18].

Antimicrobial activities of natural products from organisms have been reported with increasing interests on various fish species mucus as promising sources of antimicrobial compounds against pathogens of fish and humans [19-21]. As a naturally secreted product, mucus by fish species carries numerous functions for their survival. Mucus functions include reducing body friction against water, protection from abrasion, ventilation, ionic and osmotic control, movement, breeding, transmission, feeding, nest building, and most importantly as the first line of defense against pathogens present in the aquatic environment [22-25]. The Philippines, as an archipelago of more than 7,641 islands, has been one of the top producers of fish around the world (Fisheries & Aquaculture, 2012), due to diverse aquatic ecosystems home to different fish species that may possess potential antibiotic properties.

Contributing to the increasing body of knowledge on the search for new antimicrobials is the primary goal of the study. Hence, the study explored the potential antibacterial activity of the mucus from freshwater fish species in the Philippines against medically-important pathogens. Specifically, this sought to test the antimicrobial effects of the mucus against indicator pathogens and to elucidate potential agents in the fish mucus for drug discovery.

Materials and methods

Research design

Qualitative and quantitative research designs were applied to screen the antimicrobial activity of the mucus from selected freshwater fish species against pathogens. The research was divided into 3 phases: evaluation of the antimicrobial activity of the acidic mucus extract, the assessment of its minimum inhibitory concentration, and screening of protein component via high-performance liquid chromatography.

Specimen collection

The specimens used were reared and monitored as described by Subramanian *et al.* [26]. Live specimens of *Clarias batrachus*, *Channa striata*, and *Oreochromis niloticus* were collected from Laguna de Bay, the largest lake in the Philippines. Each fish samples were kept in a separate aquarium, equipped with aerator, maintained in ambient temperature (~29 °C). The fish samples were starved for 24 h in preparation for the mucus sample collection.

Mucus collection

Live fish samples were placed on a sterile tray and washed with 0.85 % sterile saline solution to remove any debris and contaminants associated with the epidermis. The mucus was collected aseptically at the dorsolateral region with sterile glass slides. Mucus from the ventral side was not collected as this may be contaminated with urogenital and intestinal excreta. The fishes were returned to recovery tanks every after collection. The pooled mucus was divided into 2 parts in 15 mL sterile centrifuge tubes and kept for succeeding extraction [26].

Mucus extraction

The process for acidic fish mucus extraction was done as described [26] to enhance the cationic property of the antimicrobial peptides present in the mucus. Seven milliliters (7 mL) of pooled mucus was transferred into a 15-mL centrifuge tube added with the same volume of acetic acid, and vortexed thoroughly for 30 s. The tubes were submerged in boiling water bath (100 °C) for 5 min and were cold shocked in an ice bath for 30 min. Centrifugation followed at 4000 rpm for 30 min. The supernatants were collected separately using a 10-mL sterile syringe and filtered with sterile disposable 0.22 µm pore-size polyethersulfone membrane filter unit (Whatman Puradisc® 25 AS, GE Healthcare Life Sciences, United Kingdom). The filtrate was stored in sterile screw-cap tubes at -20 °C until use.

Agar overlay diffusion assay

The antimicrobial inhibitory effects of acidic mucus extracts were tested against Gram-positive bacteria: *Enterococcus faecalis, Staphylococcus aureus*, and *Micrococcus luteus*, and Gram-negative bacteria: *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, *Escherichia coli*, and *Serratia marcescens*. The indicator strains are clinical isolates procured from the Philippine National Collection of Microorganisms - National Institute of Molecular Biology and Biotechnology (BIOTECH), University of the Philippines Los Baños. Cultures were grown in Mueller-Hinton broth at 37 °C and adjusted at 0.5 McFarland turbidity standard before the assay.

Agar-overlay well diffusion assay evaluated the antimicrobial activity of fish mucus. Fifteen milliliters (15 mL) of Mueller-Hinton soft agar mixed with 50 µL of each indicator pathogens were poured over the previously prepared Tryptic soy agar (TSA) plates. Each plate was punched with an 8-mm sterile borer. Subsequently, 100 µL of mucus extracts were added to the wells, and Cefoperazone (75 µg/disc) was used for positive control. The plates were incubated for 24 h at ambient temperature (26 - 27 °C). The antimicrobial activity was determined by observing zones of clearance around the wells and measured with a Vernier caliper. All assays were done in triplicates.

Minimum inhibitory concentration assay

Microbroth plate dilution method was used to determine the minimum inhibitory concentration (MIC) of the fish mucus extracts [27]. The MIC is described as the lowest concentration of antimicrobial agent, where here, the fish mucus extract which inhibits the growth of the pathogen.

Sterile 96-well microtiter plates were prepared for each pathogen, and 100 µL of sterile cation-adjusted MH broth was placed on columns 1 - 9 and columns 11 - 12, respectively. Column 10 was left blank intentionally to avoid contamination of the sample and the control. Briefly, 100 µL each of the mucus extracts (triplicates) were placed at row A, except for column 11. The mucus and the broth were pipetted up and down to homogenize. Subsequently, 2-fold serial dilutions were done where 100 µL was drawn from row A and were pipetted on row B and repeated upon reaching row H. One-hundred microliters (100 µL) was drawn from row H and discarded. Fifty microliters (50 µL) of standardized indicator pathogens in broth medium were added in columns 1 - 9 and 12. The titer plate was covered and incubated at 37 °C for 16 to 20 h. The assay was done in triplicates.

The lowest concentration at which the isolate was completely inhibited as evidenced by the absence of visible bacterial growth, was recorded as the minimal inhibitory concentration or MIC of the fish mucus.

High-performance liquid chromatography assay

To determine the presence of antimicrobial peptides in the selected fish mucus, the most effective epidermal fish mucus from the previous agar-overlay diffusion test was subjected to high-performance liquid chromatography (HPLC) analysis. Lysozyme at around 7 min on 280 nm and Pleurocidin at around 25 - 30 min on 215 nm were used as the reference for peak area detection as described by Cole *et al*. [28]. The HPLC machine was prepared by with a general tab, and acetonitrile injected into the HPLC system (Hitachi L-7400 UV VIS HPLC, Japan). The references for peak areas were set through the machine's software. Twenty microliters (20 µL) of catfish mucus extract was injected for analysis.

Statistical treatment of data

Data generated from each assay were recorded. Mean was used for each parameter. The results from agar-well diffusion assay were statistically evaluated using one-way ANOVA followed by Tukey's Honestly Significant Difference post hoc test to compare mean zones of inhibition for all mucus extracts and antibiotic control. All statistical analyses were performed using SPSS software. *P* values ≤ 0.05 were considered significant.

Results and discussion

Agar overlay-well diffusion assay using fish mucus acidic extract

Three freshwater fish species were sampled for the extraction of mucus and evaluated the antimicrobial activity against indicator strains. The study focused on the top aquaculture fish in the Philippines - *Oreochromis niloticus* (tilapia), *Clarias batrachus* (catfish), and *Channa striata* (snakehead fish). The results of agar overlay-well diffusion assay showed that there are statistically significant differences between the effectivity of all extracted mucus with antibiotic control, Cefoperazone, against *Aeromonas hydrophila*, *Escherichia coli*, *Klebsiella pneumoniae*, *Micrococcus luteus*, *Pseudomonas aeruginosa*, *Serratia marcescens* and *Staphylococcus aureus* as determined by one-way ANOVA at α = 0.05. Moreover, the one-way ANOVA also exhibited that there was no statistically significant difference between the effectivity of all extracted mucus and antibiotic control against *Enterococcus faecalis* (*p* = 0.546) (**Table 1**).

Table 1 Average diameter of the zone of inhibitions of mucus from Tilapia, Catfish and Snakehead Fish.

Sample	Tilapia mucus	Catfish mucus	Snakehead fish mucus	Control (Cefoperazone)	p-value
S. aureus	13.21 ± 0.54	15.53 ± 0.13	14.61 ± 0.59	36.21	0.000^{s}
E. faecalis	16.29 ± 1.44	16.20 ± 0.38	15.97 ± 0.65	17.39	0.546^{n}
P. aeruginosa	17.79 ± 0.66	19.40 ± 2.56	16.69 ± 0.57	25.16	0.016^{s}
K. pneumoniae	18.28 ± 0.74	17.81 ± 1.04	17.46 ± 0.15	30.62	0.000^{s}
M. luteus	15.32 ± 1.43	16.07 ± 2.62	15.08 ± 1.33	21.16	0.000^{s}
A. hydrophila	14.54 ± 0.52	15.73 ± 0.36	14.75 ± 0.15	20.39	0.000^{s}
E. coli	13.74 ± 0.47	15.36 ± 0.38	14.84 ± 0.52	33.16	0.000^{s}
S. marcescens	12.69 ± 0.58	15.06 ± 1.16	15.97 ± 0.89	28.12	0.000^{s}
C. tropicalis	0	0	0	37.02	0

Note: Diameter of zone of inhibition includes well diameter 8 mm.
Key:
^{s}refers to the statistically significant p-value ($p \leq 0.05$)
^{n}refers to not statistically significant p-value ($p \geq 0.05$)

A Tukey post hoc test revealed that the effectiveness of tilapia, catfish, and snakehead fish mucus are all significantly different with that of Cefoperazone against *A. hydrophila*, *E. coli*, *K. pneumoniae*, *M. luteus*, and *S. aureus*. The test also showed that the mean difference between the efficacy of catfish mucus and cefoperazone is not significant against *P. aeruginosa* (p = 0.096). Interestingly, efficacy of tilapia mucus showed significant difference between snakehead (p = 0.008) and catfish (p = 0.046) mucus against *S. marcescens* (**Figure 1**).

Among the fish mucus samples, those extracted from the catfish exhibited the highest inhibition against most bacterial pathogens, specifically against *S. aureus*, *P. aeruginosa*, *M. luteus*, *A. hydrophila*, and *E. coli.* Mucus extract from tilapia, on the other hand, exhibited highest inhibition against *E. faecalis,* and *K. pneumoniae,* and lowest against *S. aureus*, *A. hydrophila*, *E. coli*, and *S. marcescens.* Snakehead fish mucus revealed to be most effective against *S. marcescens*, and least effective against *E. faecalis, P. aeruginosa, K. pneumoniae,* and *M. luteus.* However, all fish mucus extracts were ineffective against *Candida tropicalis*.

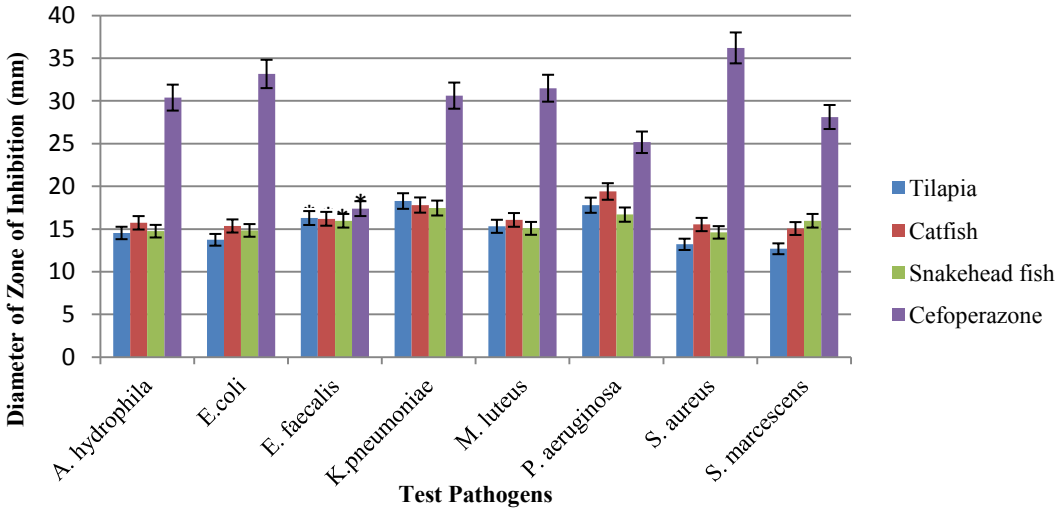


Figure 1 Comparison of the effectivity of all mucus extracts and antibiotic control (Cefoperazone) against selected pathogens. (*) means that p-values were not statistically significant (p-value > 0.05)

The prevalence of zoonotic infections from fish to humans is the primary concern for selecting *A. hydrophila*, *M. luteus, K. pneumoniae*, *S. aureus*, *E. faecalis*, *P. aeruginosa*, *E. coli*, *S. marcescens*, and *C. tropicalis*. These pathogens not only cause zoonotic infections but are causing nosocomial infections which may be acquired by immunocompromised people [29]. The mucus extracts of tilapia, catfish, and snakehead fish in this study showed an interesting bactericidal activity against bacterial pathogens which adheres with the findings of Rao *et al.* [20]. Likewise, in the one recent study, the antibacterial activity of *Arius maculatus* acidic mucus extract showed promising results [30].

Several studies revealed that the inhibitory activity of the acidic mucus extract varies depending on the nature of the indicator pathogens tested [26,31,32]. Subramanian *et al*. inferred that the variations on antimicrobial activities could be explained by the presence of inhibitory compounds present in the mucus [26]. Shepard, on the other hand, reported that the mucus-producing cells of fish secrete mucus of varying properties depending on the type of fish species [31]. While Blackstock and Pickering, and Lebedeva implied that the substances in the mucus could vary, depending on the environmental stresses such as changes in salinity or pH, and the growth and maturity of the fishes could also account for the variation of mucus activity and secretion [33,34]. Negus also stated that scale-less fishes yield a more substantial amount of mucus than scaled fishes [23].

Minimum Inhibitory Concentration assay of acidic fish mucus extract

The acidic extracts of tilapia, catfish, and snakehead fish were further analyzed for their inhibitory activities against bacterial pathogens (**Figure 2**). The results of the minimum inhibitory concentration varied for each mucus on test pathogens. *E. faecalis* revealed to be the most sensitive against tilapia mucus at a 1:4 concentration. *S. aureus* was found to be most sensitive against snakehead fish at the 1:4 concentration. Among the 3 fishes, the catfish had the highest inhibitory activity since it inhibited 2 pathogens at a 1:4 concentration compared to the activity of tilapia and snakehead fish. The results showed that the catfish mucus is effective at a 1:2 concentration against the fungi, *C. tropicalis* while both tilapia and snakehead fish are only effective at a 1:1 concentration (**Table 2**).

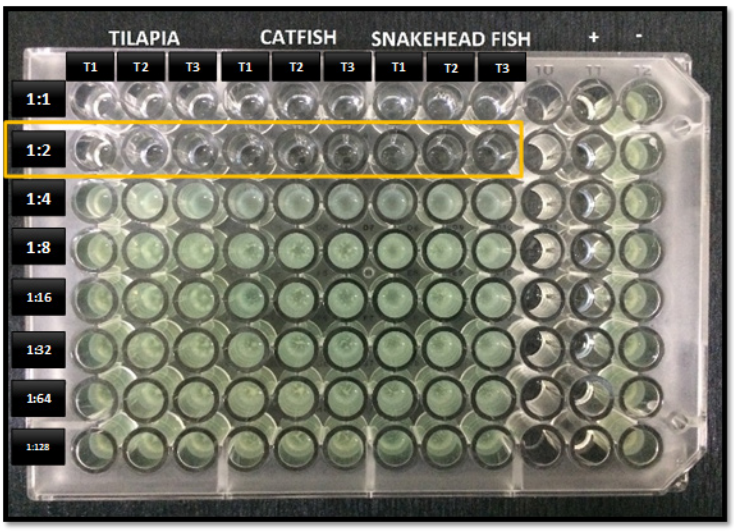


Figure 2 Minimum Inhibitory Concentration of acidic mucus extract. Rows A to H showing decreasing concentrations of acidic mucus extract. Columns 1 - 3 for *Tilapia* triplicates, columns 4 - 6 for catfish triplicates, and columns 7 - 9 for snakehead fish triplicates. Column 11 for sterile control (positive inhibition) and column 12 for growth control (negative inhibition). Yellow box indicates the minimum inhibitory concentration for each mucus extract.

The inhibitory activity of the acidic mucus extract of catfish displayed the ability to inhibit the bacteria even at a lesser concentration. The data contradict Subramanian *et al.* results wherein the mucus tested only exhibited bacteriostatic activity [26]. Moreover, Rao *et al.* showed zero activity on its acidic extract of giant snakehead and striped snakehead against *Escherichia coli*, contradicting the results exhibited by *C. striata*, where it showed bactericidal activity against the reference bacterial pathogen [20]. The effectiveness of killing the bacteria by extracting the mucus in acidic conditions was due to the heightened ability of proteins to become more soluble [35]. Ming *et al*. study suggested that acid solution enhances the solubility of cationic peptides responsible for the antibacterial activity [36]. Hancock and Lehrer furtherly stated that the large assemblage of anionic lipids on the bacterial cell surface contributes to its selectivity, thus, suggesting that higher activity of inhibiting bacterial growth is due to the enhanced solubility of cationic peptides having higher isoelectric points in acidic conditions [37].

Table 2 Results of the minimum inhibitory concentration per mucus with corresponding concentration in each bacterium inhibited.

Sample	Tilapia Mucus	Concentration	Catfish Mucus	Concentration	Snakehead fish Mucus	Concentration
S. aureus	++	1:2	++	1:2	+++	1:4
E. faecalis	+++	1:4	+++	1:4	++	1:2
P. aeruginosa	++	1:2	++	1:2	++	1:2
K. pneumoniae	++	1:2	++	1:2	++	1:2
M. luteus	++	1:2	+++	1:4	++	1:2
A. hydrophila	++	1:2	++	1:2	++	1:2
E. coli	++	1:2	++	1:2	++	1:2
S. marcescens	+	1:1	++	1:2	++	1:2
C. tropicalis	+	1:1	++	1:2	+	1:1

Key: (+) – Least concentration to inhibit bacterial growth is 1:1
(++) – Least concentration to inhibit growth is 1:2
(+++) – Least concentration to inhibit bacterial growth is 1:4

Detection of antimicrobial peptides using high performance liquid chromatography assay

The top-performing mucus extract from catfish was analyzed for the detection of antimicrobial peptides based on the method of Cole *et al.* using high-performance liquid chromatography [28]. Absorbance at 215 nm was used as reference for pleurocidin and absorbance at 280 nm for lysozyme at specific retention times. The results of the HPLC revealed various high peaks at different retention times. Highest peaks for the detection of pleurocidin was at 14.93 and 20.79 min with concentrations of 11.702 and 13.825, respectively, while for lysozyme detection (**Figure 3**), highest peaks showed at about 6.73 and 7.62 min with concentrations of 40.245 and 26.243, respectively. These results however do not signify that the peptides present are of pleurocidin and lysozyme as there may be of other peptides present in the extract as well [28]. Various journals were reviewed and used as references to determine the retention time for peptide separation and peaks [28,38]. A general column was used for the chromatography assay.

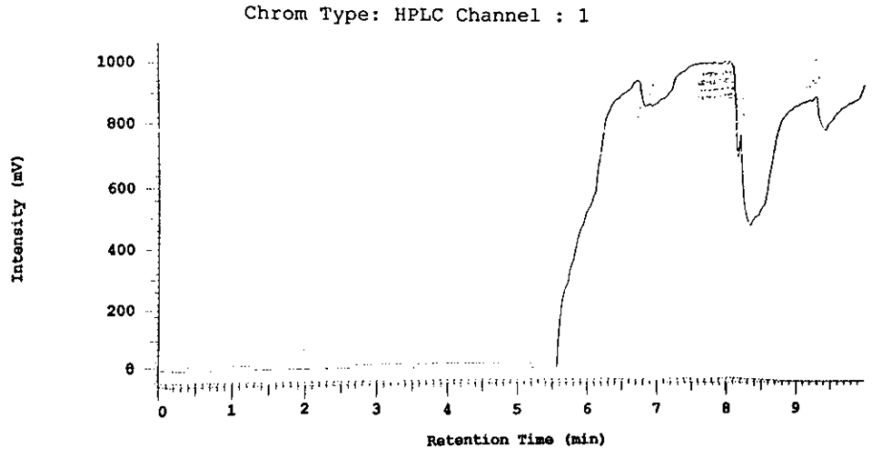


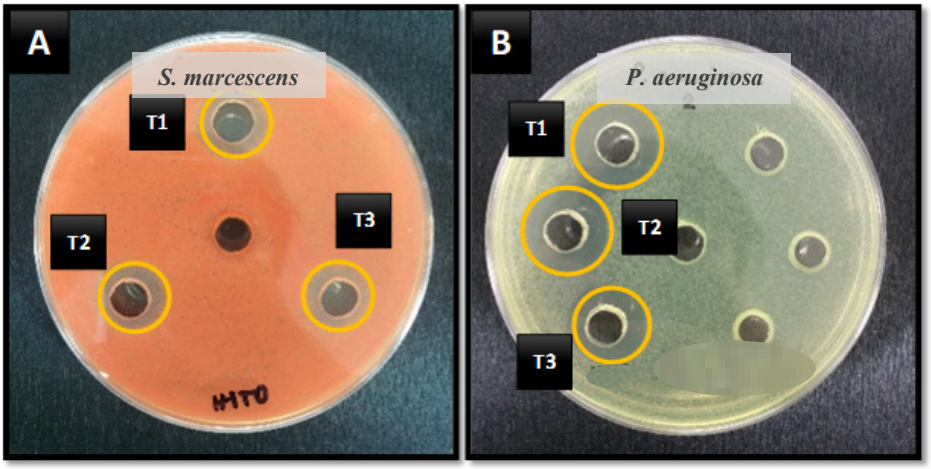
Figure 3 Detection of Lysozyme at a wavelength of 280 nm with a retention time of 10 min. Lysozyme estimated peaks at 7 min.

Such peptides; paradaxins, proteases, lectins, pleurocidins, and lysozymes in fish mucus have been detected based on several related studies where potential antimicrobial activity against a wide array of pathogens was observed [39,40,28]. These studies became the basis of choosing pleurocidin and lysozyme as reference for the HPLC assay. Notably, antimicrobial peptides (AMPs) such as lysozyme and pleurocidin show a promising broad-spectrum of antimicrobial activity due to its ability to have specific modes of action in the formation of pores in the membrane. Limited attempts were conducted to determine the ability of different AMPs to inhibit bacterial pathogens, most especially against multidrug-resistant strains. Pleurocidins are usually found to be present in different mucosal membranes such as the skin, gut, and gills [41]. Lysozymes, on the other hand was found to have a possible synergistic effect with pleurocidin [42]. The expressions of AMPs in fish mucus have been demonstrated in several studies and revealed that the antimicrobial property is enhanced when the fish is subjected to different environmental conditions including exposure to high microbial environment [40,43-45].

Further screening of acidic fish mucus extract against multi-drug resistant pathogens

The antimicrobial activity of fish mucus extracts was tested against multi-drug resistant bacteria: MBL-*Pseudomonas aeruginosa*, ESBL-*Escherichia coli*, and Methicillin-Resistant *Staphylococcus aureus* (**Figure 4**). The results of agar overlay-well diffusion assay showed a statistically significant difference between the inhibitory effects of all extracted mucus and antibiotic control, Cefoperazone, against MBL-*Pseudomonas aeruginosa*, ESBL-*Escherichia coli*, and Methicillin-Resistant *Staphylococcus aureus* as determined by one-way ANOVA at 0.05 significance level (**Table 3**).

A Tukey post hoc test revealed the antimicrobial effects of tilapia, catfish, and snakehead fish mucus are all significantly different with that of Cefoperazone against all multidrug-resistant bacteria. The efficacy of catfish mucus showed a significant difference in the efficacy of both catfish, and snakehead fish against MBL-*P. aeruginosa*. It was observed that among the mucus samples, those extracted from the snakehead fish exhibited the highest inhibition against all multidrug-resistant bacterial pathogens. Tilapia mucus extract showed the least effects against ESBL-*E. coli* while catfish mucus extract showed lowest inhibitory effects against MBL-*P. aeruginosa.* Though results imply fish mucus antimicrobial activity varies depending on the fish origins of the mucus and the target test organism, hence, surprisingly exhibited antibacterial effects against drug-resistant strains.



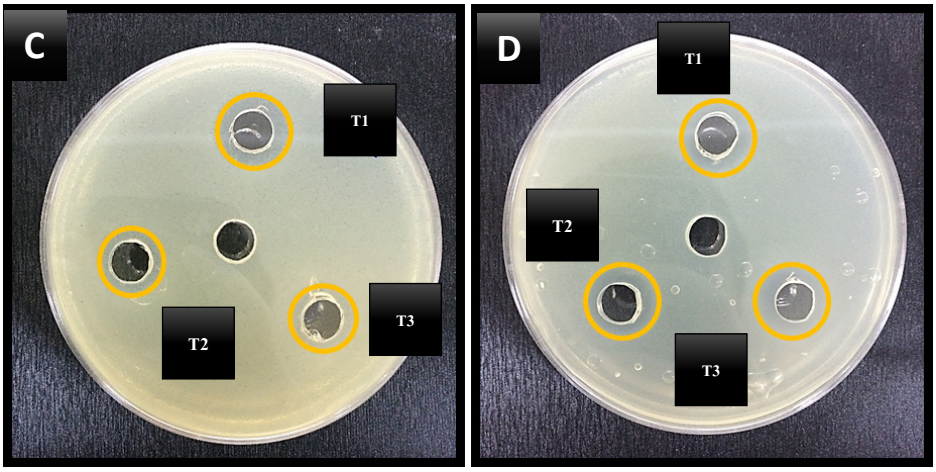


Figure 4 Agar overlay-well diffusion assay results of epidermal fish mucus. Each 8mm wells contained 100 µL of mucus and tested against indicator strains; A.*Serratia marcescens*, B. *P. aeruginosa*, C. Metallo-β-lactamase (MBL)-*Pseudomonas aeruginosa*, and D. Extended Spectrum Beta-Lactamase (ESBL)-*Escherichia coli*. Zones of inhibitions (yellow circles) were observed after 24 h of incubation.

Table 3 Average diameter of the zone of inhibitions of mucus from Tilapia, Catfish, and Snakehead fish against multidrug resistant bacteria.

Sample	Tilapia Mucus	Catfish Mucus	Snakehead fish Mucus	Control (Cefoperazone)
MBL-*Pseudomonas aeruginosa*	19.07 ± 2.02	15.50 ± 0.95	20.12 ± 1.27	NI
ESBL-*Escherichia coli*	14.18 ± 1.00	15.36 ± 0.13	15.43 ± 0.87	17.39
Methicillin-Resistant *Staphylococcus aureus*	13.90 ± 0.78	13.98 ± 0.90	14.21 ± 0.27	NI

Note: Diameter of zone of inhibition includes well diameter 8 mm.
Key: NI = no inhibition

Conclusions

Fish mucus has been studied as promising source of natural antimicrobial components. Here, the antimicrobial activity of fish mucus of economically important freshwater fish species in the Philippines - *Oreochromis niloticus* (tilapia), *Clarias batrachus* (catfish), and *Channa striata* (snakehead fish)- against fish and human pathogens was investigated. Based on the findings, fish mucus extracts exhibited antibacterial activity against *A. hydrophila*, *E. coli*, *K. pneumoniae*, *M. luteus*, *P. aeruginosa*, *S. marcescenes*, *E. faecalis*, *S. aureus*, MBL-*P. aeruginosa*, ESBL-*E. coli*, and Methicillin-Resistant *S. aureus* clinical strains. Among all the samples, mucus extracted from catfish demonstrated the highest inhibitory effects at the lowest concentration against Gram-positive *S. aureus* and *M. luteus*. Though fish mucus extracts did not exhibit antifungal activity against *C.tropicalis*, still exhibit high antibacterial effects. The presence of lysozyme and pleurocidin, which are known antimicrobial proteins, have been detected in the fish mucus sampled. Three species of freshwater fish were used for mucus extraction, but have revealed highest activity by catfish mucus. The investigation revealed that the antibacterial effects of fish mucus varies across species of fish. Antimicrobials employ different mechanisms of action against bacterial species specifically on targeting cell-membrane, where here, fish mucus showed high activity

mostly against Gram-positive bacteria, supporting nature of antimicrobial effects of detected lysozyme and pleurocidin in the fish samples. It is empirical to unravel the influence of other variables which may contribute to the increased antibacterial effects of fish mucus; extraction method, and influence of environmental stresses on the expression of antimicrobial peptides in the mucus. Future studies shall explore the suitability of catfish mucus in antibacterial studies addressing the need for finding novel compounds for the development of new antimicrobial drugs.

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