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Antibacterial and Bio-active Component analysis by GC-MS of Epidermal mucus of (snakehead) *Channa striata* L. from Freshwater Bodies of India

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Abstract:
In reaction to the macro and microenvironment, the fish skin mucus has a unique immune role. The goal of the current study was to characterise the biochemical properties of the epidermal mucus of the freshwater striped snakehead *Channa striata* L. as well as its antibacterial capacity. In order to do this, epidermal mucus samples were collected and tested for their ability to inhibit the growth of four pathogenic bacterial species, including *Staphylococcus aureus*, *Bacillus subtilis*, *Pseudomonas aeruginosa*, and *Escherichia coli*. According to the findings, *C. striata* mucus had a stronger antibacterial impact against *Pseudomonas aeruginosa* and *Escherichia coli*. Results of GC-MS analysis indicates the presence of some bioactive compound like Silane, dimethoxymethyl-, Silanediol, dimethyl-, Cyclotrisiloxane, hexamethyl-,Cyclotetrasiloxane, octamethyl-,Cyclopentasiloxane, decamethyl-,Azulene, Cyclohexasiloxane, dodecamethyl-, 2,4-Di-tert-butylphenol, Cyclooctasiloxane, hexadecamethyl- etc. Thus, the present study confirmed that *C. striata* epidermal mucus is having potentials of antibacterial activity. However, the study offers the baseline information for the pharmaceutical industry's new medicine development.

Keywords:
Antibacterial activity, Bioactive components, Epidermal mucus, *Channa striata* (Snakehead), GC-MS analysis.

1. Introduction:

A complex interaction between aquatic creatures and bacteria defines life in the aquatic ecosystem. Numerous pathogenic bacteria and aquatic life, including fish, are involved in this relationship. The contact could lead to the necessity for protection arises from exposing these aquatic species to harmful toxins [Al-Rasheed *et al*., 2018]. Fish mucus is regarded as being more beneficial than other fish by-products and has antibacterial proteins. It is also considered as a crucial element of innate immunity and functions to stop the colonisation of the organism by bacteria, fungus, and parasites [Wei *et al*., 2010]. The epidermal goblet cells of fish secrete fish mucus, which is made up of mucins and other materials such proteins, lipids, immunoglobulin, inorganic salts, and oils suspended in water. The fish's surface mucus layer is regularly changed, presumably preventing persistent colonisation by bacteria, fungus, and parasites. Numerous polypeptides with antibacterial characteristics can be found in skin secretions. Proteases are thought of as antimicrobial proteins that are involved in the control of antimicrobial peptide synthesis [Bragadeeswaran *et al*., 2011]. Nature has consistently been the primary source for the discovery of novel bioactive molecules and pharmaceuticals, which are crucial for combating infections and a variety of disorders, throughout the history of drugs [Patel *et al*., 2020]. Recently, fish have also been thought of as a potential new source of therapies, nutraceuticals, pharmaceuticals, and functional meals [Alshammari *et al*., 2020]. It is a staple meal and a key source of the micronutrients needed to avoid malnutrition and vitamin and mineral deficiencies in rural areas. Despite being recognised by current and previous medical supplies worldwide and being well known as a constituent in many famous medications, snakeheads have not yet been completely studied as a source of medicines [Rahman *et al*., 2018]. Multiple fish species have shown fish mucus to have antibacterial properties. Yet this activity seems to vary from species to species the *Puntius sophore*, climbing perch (*Anabas testudineus*), Ray species (*Dasyatis marmorata* and *Gymnura altavela*), *Cynoglossus arel* and *Arius caelatus* can be specific towards the bacteria. However as per our knowledge no study has been reported detailing the antibacterial and bio-active component analysis of the *C. striata* skin mucus extract to date. The goal of the current study was to look into and evaluate antibacterial activity and try to found some bio-active component analysis by GC-MS of epidermal mucus of *C. striata*.

2.Materials and Methods

2.1Fish for mucus collection

Healthy and adult walking snakehead (*Channa striata*) L. were produced from local fish market in Nanpura Surat, (21.19085N,72.81980E) The collected fishes were then transferred to laboratory and placed in to fish tank to acclimatize for 5 days.

2.2External mucus collection

Mucus collection was done with certain modifications with following the method of [Subramanian *et al*., 2018]. Mucus was obtained from 3 fish representing for sample collection. No anesthetic chemical treatment was given to selected fish before collection of mucus sample. Mucus sample was carefully scraped from the dorsal surface of fish body by the usage of a sterile spatula and collected into sterile container. Then the fish were returned into the recovery tanks. Collected fish mucus sample was stored in refrigerator at 4°C until further use.

2.3Extraction

The pooled mucus sample was further divided into two portions to prepare crude extract, acid extract and methanolic extract.

2.3.1Crude extract: For the crude extract 10 mL mucus was homogenized by using a polytron homogenizer then centrifuged it at 10,000 rpm for 30 min at 4°C. Supernatant was collected and filtered with Watman No.1 filter paper and 0.45µm Millex, syringe filter units, 33 [Hellio *et al*., 2002]. Prepared crude extract was stored at 4°C until further use.

2.3.2Acid extract: To prepare an acidic extract, 10 mL of pooled the mucus sample were combined with 10 mL of 10% acetic acid, and the mixture was then heated for 5 min in a water bath. The mixture was then centrifuged for 30 min at 4°C using 10,000 rpm. Watman No. 1 filter paper and 0.45-µm Millex syringe filters were used to collect and filter the supernatant. To get a 2000 g/mL concentration, the final dry extract was re-suspended in deionized water. Prepared mucus extract was prepared and kept at 4°C until further use.

2.3.3 Methanol extract: The mucus was collected in methanol, homogenized with a polytron homogenizer, and centrifuged at 10,000 rpm for 30 min at 4°C to make the methanol extract. Watman No. 1 filter paper and 0.45µm Millex syringe filter units were used to collect and filter the supernatant. The prepared methanolic mucus extract was kept at 4°C for further use.

2.4 Determination of Minimum Inhibitory Concentration (MIC)

Utilizing Mueller Hinton broth (MHB) and following the guidelines established by the Clinical and Laboratory Standards Institute (CLSI) with minor modifications, the minimum inhibitory concentration (MIC) was determined using the serial dilution technique to assess antibacterial activity. Bacterial inoculums were prepared in MHB and spent a 24 h period at 37°C. The mucus extract was subjected to two-fold dilution ranging from 2000 to 0.48 µg/ml (with a final volume of 80 µl) in phosphate buffer saline. The absorbance of each well was determined using an EpochTM microplate spectrophotometer at 600 nm. Plates were then incubated at 37°C for 24 h. After incubation, the absorbance was read again at the same wavelength and the obtained absorbance values were subtracted from those initial values before incubation, following the protocol outlined by the [CLSI 2014]. Assessment was conducted simultaneously for bacterial growth control (MHB + bacteria + mucus extract vehicle) and sterility control (MHB + bacteria + mucus extract vehicle), with streptomycin used as the positive control. MICs were recorded as the lowest concentration that inhibited bacterial growth.

2.5 Antibacterial assays

2.5.1 Bacterial strains

The strains utilized in this study comprised two Gram-positive bacterial strains: *Bacillus subtilis* (MTCC 121) and *Staphylococcus aureus* (MTCC 96), as well as two Gram-negative bacterial strains: *Escherichia coli* (MTCC 9537) and *Pseudomonas aeruginosa* (MTCC 741). These strains were obtained from the Microbial Type Culture Collection and Gene Bank (MTCC) located at the Institute of Microbial Technology, Shanti Path, 39A, Sector 39, Chandigarh.

2.5.2 Agar cup diffusion assay

The mucus extract was assessed using the agar cup/well diffusion method. All bacterial strains were uniformly spread (100 µl) over the agar plates and wells were punctured using a gel puncture. Subsequently, 100 µl of mucus extract (2000 µg/ml) was inoculated into each respective well. The plates were then incubated at 37°C for 24 h, following the protocol outlined by [Pradhan *et al*., 2022]. On the following day, the zone of inhibition was measured

using a scale, extending from the center to the edge of the inhibition zone. For the positive control, Streptomycin standard antibiotic was utilized. All the experiment were carried out I triplicates. Data represented as mean ± SD.

2.6 GC-MS analysis

For GC-MS analysis, 1.0 µl of methanol extract was injected split mode using a SHIMADZU GC Nexis GC-2030 system equipped with a column measuring 30m × 0.25 mm 0.25 µm film thickness. The inlet temperature was set at 250°C at 5°C min-1. Helium was utilized as the carrier gas at a stable flow rate of 1.0 mL/min. The column temperature was initially maintained at 50°C for 1 min before being ramped up to 240°C at a rate of 10°C min-1, followed by an additional increase at 20°C min-1 to reach 240°C, where it remained for 4 min. The column effluent was introduced into the ion source at a temperature at 270°C. The mass spectrometer was operated in electron impact (EI) mode at 70 ev. Data acquisition was conducted in full scan mode from m/z 35 to 500 with a scan time 0.3 s. GC-MS analysis utilized electron impact ionization at 70ev and data were evaluated using total ion chromatogram (TIC) for compound recognition and quantification [Uyan et al., 2020]. The spectra of the components were compared with a database of known component spectra stored in the GC-MS library.

3. Results and Discussion

In this work, epidermal mucus was tested for antibacterial activity using three distinct extraction methods. For the epidermal mucus of *C. striata*, a crude, acidic and methanol extract were prepared. GC-MS analysis was performed by using methanol extract.

3.1 Antibacterial assay

The MICs values are represented in Table 1 while a summary of the zone of inhibition is provided in Table 2 and Figure 1. These findings suggest that the mucus extract of *C. striata* displayed inhibitory activities against all tested pathogens. The antibacterial activity of the mucus extract from *C. striata* was assessed against both Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative (*P. aeruginosa* and *E. coli*) bacteria using the agar cup/well diffusion method. The results of the antibacterial activity were presented in terms of the zone of inhibition observed against all four tested bacterial strains. Notably, *P. aeruginosa* and *S. aureus* demonstrated greater susceptibility compared to *B. subtilis* and *E. coli* Figure 1. These findings suggest that the mucus extract of *C. striata* displayed inhibitory activities against all tested pathogens. Fish epidermal mucus has been shown in several previous investigations to possess strong antibacterial activities against a wide range of microbiological infections [Subramanian *et al.,* 2008; Balasubramanian *et al.,* 2012]. Studies conducted in the past by [Hancock and Sahl 2006] revealed that extracting fish mucus in an acidic environment increases the solubility of proteins, which may increase the antibacterial efficiency of the extracts. Presence of lysozyme is also one of the enzymes that increases bactericidal activity, acidic mucus extracts showed the highest levels of bactericidal activity [Wei *et al*., 2010; Venilla *et al.,* 2011]. Additionally, a number of studies have found antimicrobial peptides in epidermal mucus that shows bactericidal effects [Valero *et al*., 2013]. These peptides could be essential for the antibacterial action that has been seen, highlighting the diverse range of antimicrobial chemicals that epidermal mucus may contain. Thus, our research implies that lysozyme may not be the only substance in epidermal mucus that has bactericidal action; other molecules within the mucus may also play a role.

3.2 GC-MS analysis

GC-MS analysis was performed, utilizing methanol extract, of the epidermal mucus of *C. striata* in the biochemical analysis; twenty-six compounds were identified. Among these,

major bioactive compounds in the mucus of *C. striata* were identified as Silane, dimethoxymethyl-(19.56%), Silanediol, dimethyl-(9.39%), Cyclotrisiloxane, hexamethyl-(2.15%), Cyclotetrasiloxane, octamethyl-(2.25%), Cyclopentasiloxane, decamethyl-(1.01%), Azulene (35.06%), Cyclohexasiloxane, dodecamethyl-(5.85%), 2,4-Di-tert-butylphenol (2.34%), Cyclooctasiloxane, hexadecamethyl-(4.3%). Figure 2 presents the chromatogram of the GC-MS profile for methanolic epidermal mucus. The peaks in the chromatogram were integrated and compared to the database spectra of known components stored in the GC-MS Library, as outlined in Table 3.

Overall, our research shows that the crude and acidic epidermal mucus extraction from *C. striata* demonstrated broad-spectrum bactericidal efficacy against the pathogenic bacteria that were examined. Fish mucus contains a variety of bioactive components, which are probably responsible for the antibacterial activity that has been found. Consequently, antibacterial substances found in fish epidermal mucus have the potential to replace antibiotics in aquaculture procedures. Antibiotic resistance in aquaculture may be mitigated by using epidermal mucus as a natural product.

4. Conclusion

The aim of this study was to found that epidermal mucus of the *C. striata* give the antibacterial effect or not and also found that epidermal mucus has some bio-active compound that gives the antibacterial, antioxidant, anticancer activity. The results of the work shows that epidermal mucus in acid extract gives the antibacterial activity against both gram-negative and gram-positive bacteria and on the basis of GC-MS of methanolic extract findings shows that mucus have some bioactive compound that gives difference type of biological activity. This study finds that epidermal mucus of *C. striata* use as a pharmaceutical product against different type of pathogens, cancer disease etc.

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Table 1: MIC results of crude and acid extract against pathogenic bacteria

Pathogen	**MIC (µg/ml)**	
	C. striata	
	Crude extract	**Acid extract**
Staphylococcus aureus (MTCC-96)	0.04	0.55
Bacillus subtilis (MTCC-121)	0.03	0.31
Pseudomonas aeruginosa (MTCC-741)	0.048	0.62
Escherichia coli (MTCC-9537)	0.035	0.40

Table 2: Zone of inhibition of antibacterial activity

Pathogens	**Zone of Inhibition in (mm)**		
	Antibiotic	**Crude extract**	**Acidic extract**
S. aureus	12.2	0.30	09.3
B. subtilis	12.5	0.35	08.8
P. aeruginosa	12.0	0.36	09.5
E. coli	12.7	0.4	08.6

Table 3: GC-MS analysis of Epidermal mucus of *Channa striata*

R. Time	Peak Area%	M.W	M.F	Name of identified compound	Biological activity	References
2.504	19.56	106	C3H9O2Si	Silane, dimethoxymethyl-		Femi-Adepoju *et al*., 2018
2.823	9.39	92	C2H8O2Si	Silanediol, dimethyl-	Bactericidal, Antimicrobial, HIV protease inhibitor	TL *et al*., 2021
3.057	4.65	118	C6H14O2	Ethane, 1,1-diethoxy-		
3.552	1.27	118	C6H14O2	2,2-Dimethoxybutane	antibacterial activity	
5.007	2.15	222.46	C6H18O3Si3	Cyclotrisiloxane, hexamethyl-	Antimicrobial, antioxidant, antibacteria	Ismail *et al*., 2019
6.768	0.95	132.28	C6H16OSi	Silane, (2-methoxyphenyl)trimethyl-		
8.081	1.81	73.14	C4H11N	Ethanamine, N,N-dimethyl-	Antibacterial and antifungal activity	
8.644	0.4	382.4	C20H30O7	Benzoic acid, 2-formyl-4,6-dimethoxy-, 8,8-dim		
9.444	2.25	296.61	C8H24O4Si4	Cyclotetrasiloxane, octamethyl-	Antimicrobial, antioxidant, antibacterial	Ismail *et al*., 2019
10.42	0.9	136.23	C10H16	D-Limonene	antibacterial, antifungal, and antiviral	
11.7	0.62	138.12	C7H6O3	2,5-Dihydroxybenzaldehyde, 2TMS derivative	antioxidant activity	
11.78	0.74	170.33	C12H26	Dodecane	Antibacterial and antifungal activity	
12.39	1.01	370.77	C10H30O5Si5	Cyclopentasiloxane, decamethyl-	antioxidant activity	Kadri et al., 2011
13.32	35.06	128.17	C10H8	Azulene	anti-inflammatory and peptic ulcers, antineoplastic with leukemia, antidiabetes, antiretroviral with HIV-1, antimicrobial photodynamic therapy, and antifungal activity	Bakun *et al*., 2021
13.91	0.32	140.14	C5H8N4O	5-Amino-1-methyl-1H-pyrazole-4-carboxamide		
14.64	0.25	226.44	C16H34	Hexadecane	Antibacterial, antioxidant activities	kumari *et al*., 2019
15.01	5.85	444.92	C12H36O6Si6	Cyclohexasiloxane, dodecamethyl-	Antibacterial and cytotoxicity activity	Thambidurai *et al*., 2017
16.05	0.68	184.57	C8H5ClO3	5-Chloro-N-(3-cyanophenyl)-2-hydroxybenzen	Anti-inflammatory and analgesic, Anticancer, Anti-HIV, Antioxidant, Antimicrobial, Antimalarial	kumar and Ritika *et al*., 2020
16.41	0.91	198.39	C14H30	Tetradecane	Antibacterial and antifungal activity	Nasr *et al*., 2022
16.53	0.38	226.4	C15H30O	Pentadecanal-		
17.27	3.3	519.07	C14H42O7Si7	Cycloheptasiloxane, tetradecamethyl-	Antioxidant, flavor, hypocholesterolemic agent	Erdoğan *et al*., 2020
17.8	2.34	206.32	C14H22O	2,4-Di-tert-butylphenol	Antifungal, antioxidant activity	Varsha *et al*., 2015

18.95	0.93	254.5	C18H38	Octadecane	Enzymic activity	Erdoğan *et al*., 2020
19.27	4.3	593.2	C16H48O8Si8	Cyclooctasiloxane, hexadecamethyl-	Antimicrobial, antioxidant, antibacterial	Ismail *et al*., 2019

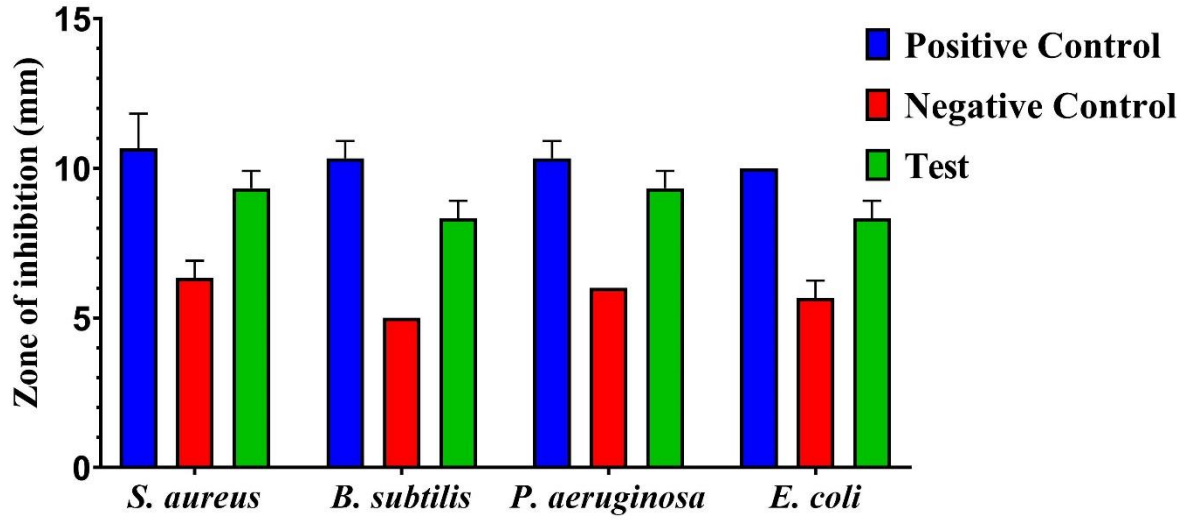

Figure 1: Antibacterial activity against *S.aureus, B.* subtilis, *P.aeruginosa* and *E.coli.* All experiment were carried out in triplicate, and data represent the mean ± SD. Statistical significance was determined using ANOVA t-test (*p <0.05). Positive control= Antibiotic, Negative control=10% Acetic acid, Extract= Acid Extract

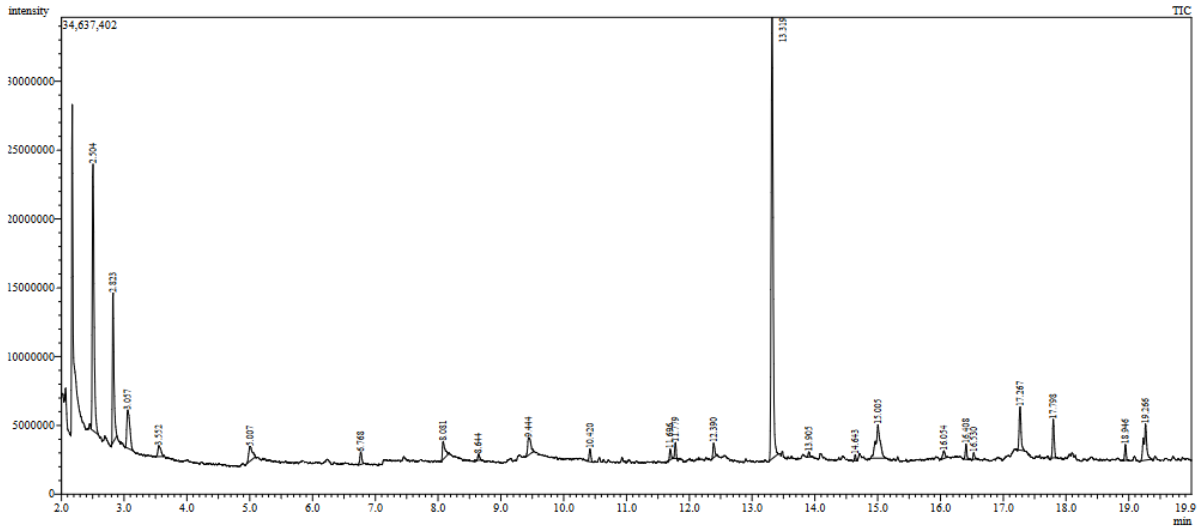

Figure 2: GC-MS analysis indicates the presence of some bioactive compound like Silane, dimethoxymethyl-, Silanediol, dimethyl-, Cyclotrisiloxane, hexamethyl-,Cyclotetrasiloxane, octamethyl-,Cyclopentasiloxane, decamethyl-,Azulene, Cyclohexasiloxane, dodecamethyl-, 2,4-Di-tert-butylphenol, Cyclooctasiloxane, hexadecamethyl- etc.