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Isolation, Biochemical Characterization And Antimicrobial Studies On Skin Mucus Of Fresh Water Spiny Eel Macrognathus Siamensis

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Abstract:
Background*:* Macrognathus siamensis *is an economically and medicinally important freshwater spiny eel whose epidermal mucus plays a crucial role in protection and innate immunity. The present study investigated the biochemical composition of lyophilized epidermal mucus and evaluated its antimicrobial potential. The mucus was found to contain bioactive components, including proteins and carbohydrates, known to contribute to defense mechanisms. Antimicrobial assays demonstrated significant antibacterial and antifungal activities against selected human and fish pathogens. These activities are attributed to the presence of antimicrobial peptides and other proteinaceous compounds in the mucus. The findings highlight the multifunctional nature of* M. siamensis *mucus and its potential application as a natural source of antimicrobial agents*.
Materials and Methods*: The present study investigated the physicochemical properties, biochemical composition, and antimicrobial potential of epidermal mucus from the freshwater spiny eel* Macrognathus siamensis*. Live specimens were collected from the Cauvery River, acclimatized under laboratory conditions, and epidermal mucus was carefully harvested and lyophilized for analysis. Physicochemical characterization included solubility, pH, electrical conductivity, and melting point, while biochemical constituents were evaluated using carbohydrate and protein assays, FTIR, HPLC, and UV–Visible spectroscopy. Multiple solvent extracts of mucus were prepared to assess bioactive potential. Antibacterial activity was evaluated against selected human and fish pathogens, and antifungal activity against common fungal strains using agar diffusion assays. The results demonstrated the presence of bioactive compounds with broad-spectrum antimicrobial activity, highlighting the role of* M. siamensis *mucus in innate defense and its potential application as a natural antimicrobial source.*
Results*: The epidermal mucus of* Macrognathus siamensis *exhibited distinct physicochemical properties, including polar solubility, slightly acidic pH, measurable ionic conductivity, and thermal stability associated with carbohydrate content. Biochemical analyses confirmed the presence of carbohydrates, proteins, and lipids, supported by FTIR, HPLC, and UV–Visible spectral profiles indicating bioactive functional groups and metabolites. The mucus demonstrated broad-spectrum antibacterial activity against human and fish pathogens, with Extract D showing the highest inhibitory effect, surpassing ampicillin. Significant antifungal activity was also observed against* Aspergillus *spp. and* Candida albicans*. Overall, the results highlight the mucus as a rich source of bioactive compounds with potent antimicrobial potential.*
Conclusion: *The epidermal mucus of* Macrognathus siamensis *is rich in bioactive molecules that contribute to its physicochemical stability and innate immune defense. Its broad-spectrum antibacterial and antifungal activities demonstrate strong protective potential against diverse pathogens. These properties highlight the mucus as a promising natural source for antimicrobial agents and sustainable applications in aquaculture health management.*
Key Word*:* Macrognathus siamensis*, FTIR, HPLC, Antibacterial and antifungal activity.*

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I. Introduction

Macrognathus siamensis is a freshwater spiny eel widely distributed across South and Southeast Asian freshwater ecosystems and is recognized as an economically important food fish due to its high nutritional value, particularly protein and omega-3 fatty acids, which contribute to cardiovascular health (Gulzar & Zuber, 2000). Beyond its dietary importance, *M. siamensis* is traditionally regarded as medicinally valuable. The skin of *M. siamensis* secretes substantial mucus, constituting approximately 0.5–1.0% of body weight, which surpasses many teleost fishes. The epidermis serves as the primary interface between the fish and its environment, acting as the first line of defense against physical injury, pathogens, and environmental stressors (Pickering et al, 1974). The mucus layer, secreted by goblet cells, forms a protective cuticle over the epidermis

(Anbuchezhian et al., 2011). Club cells secrete noxious substances functioning primarily as antipredator deterrents and contribute to immune defense (Knouft et al, 2003).

Fish mucus is a viscous secretion composed mainly of mucins, along with inorganic salts, lipids, immunoglobulins, and other bioactive molecules, providing lubrication and forming a biochemical barrier against pathogens (Pearson & Brownlee, 2005). High-molecular-weight mucins occur in monomeric and oligomeric forms, with oligomers responsible for the gel-like consistency of mucus (Bauer et al, 1966; Thornton & Sheeham, 2004). Mucins and associated bioactive compounds exhibit antimicrobial, antifungal, antiviral, and antipredator activities, contributing to innate immune defense (Knouft et al, 2003).

Epidermal mucus also prevents pathogen colonization, as microbial adhesion to mucus is a key step in host infection (Yan et al, 2010). Fish mucus participates in multiple physiological processes, including respiration, ionic and osmotic regulation, reproduction, locomotion, and defense against environmental perturbations (Shephard, 1994). Antimicrobial peptides (AMPs), present in mucus, serve as a first line of defense against invading pathogens (Boman, 1995). Overuse of synthetic antimicrobials has prompted interest in natural alternatives, and several studies have reported antibacterial and antifungal activities in teleost mucus, with proteinaceous secretions containing AMPs forming an integral component of host defense (Balasubramanian et al, 1995).

The present study aimed to investigate the biochemical composition of *M. siamensis* epidermal mucus, focusing on antimicrobial activities of lyophilized mucus extracts. The findings provide insights into the multifunctional role of mucus in innate immunity and its potential applications as a source of bioactive compounds.

II. Material And Methods

Collection and acclimatization of fish

Live specimens of spiny eel, *Macrognathus siamensis*, were collected from selected locations of the Cauvery River, Erode District, Tamil Nadu, India, during the study period. The geographical coordinates of the sampling sites were recorded using a Global Positioning System (GPS). The collected fishes were transported to the laboratory and acclimatized in artificial cave-fitted fiberglass tanks under controlled laboratory conditions with continuous aeration and regular water exchange.

Sample Collection and Preservation

Epidermal mucus was carefully scraped from the dorsal surface using Whatman filter paper, avoiding ventral regions to prevent urinal or spermal contamination. The mucus was immediately mixed with methanol:water (2:1, v/v), centrifuged at 3,000 rpm for 10 min. The supernatant was collected and lyophilized at the DRDO Centre for Life Sciences, Bharathiar University, Coimbatore, India. The lyophilized mucus powder was stored at −4 °C until further analysis.

Solubility

Powdered mucus (0.05 g) was separately dissolved in polar and non-polar solvents, including Milli-Q water, acetonitrile (ACN), trifluoroacetic acid (TFA), methanol, and chloroform, to assess solubility properties.

pH Measurement

Five milligrams of mucus were dissolved in 2 mL Milli-Q water. A pH meter was calibrated with acetate (pH 4) and ammonium (pH 10) buffers. After rinsing and drying the electrodes, the pH of the mucus solution was recorded.

Electrical Conductivity

0.08 g sample of mucus was dissolved in 10 mL Milli-Q water. The conductivity meter was standardized using 0.05235 g KCl in 100 mL distilled water. Conductivity of the mucus solution was measured and recorded.

Melting Point

The melting point of powdered mucus was determined using a capillary tube in a melting point apparatus. The temperature at which the sample liquefied was noted.

Biochemical Analysis

Carbohydrate Estimation

Total carbohydrate was determined using the anthrone method (Seifter et al, 1950). Sample homogenates were mixed with 2 mL anthrone reagent, incubated in a boiling water bath for 15 min, cooled in the dark, and absorbance measured at 750 nm. Glucose (100 mg/100 mL) was used as standard.

Protein Estimation

Total protein was estimated by the Lowry method (Lowry et al, 1951). Samples were homogenized with 80% ethanol, centrifuged at 5,000 rpm for 15 min, and the precipitate dissolved in 1 M NaOH. An aliquot (0.5 mL) was reacted with solution C and Folin–Ciocalteu reagent, and absorbance measured at 640 nm.

FTIR Spectroscopy

Powdered mucus (5 mg) was analyzed by FTIR (Nicolet 6700) over 4000–500 cm^{-1} at 25 ± 1 °C. Spectra were processed using OriginPro 8.0 to identify functional groups.

HPLC Analysis

Biochemical composition was determined using an Agilent 1220 Infinity HPLC system with a C18 column. Solvent A was Milli-Q water with 0.5 mL phosphoric acid, and solvent B was acetonitrile. Analysis was performed over 35 min. Samples (0.05 g mucus in 0.5 mL water) were filtered and injected. Peaks were recorded and calibrated against standards.

UV-Visible Spectrophotometry

Two hundred milligrams of mucus dissolved in 8 mL Milli-Q water. Absorbance spectra were recorded using a Specord S 100 spectrophotometer and analyzed with OriginPro 8.0.

Antimicrobial activity

Preparation of mucus extracts

- ***Extract A (Aqueous extract)*****:** One milligram of lyophilized mucus sample was dissolved in 1 mL of phosphate buffer solution.
- ***Extract B (Acetic acid extract)*****:** One milligram of lyophilized mucus was boiled in 1 mL of 10% acetic acid for 5 minutes, cooled, and centrifuged at 18,000 rpm for 35 minutes at 4 °C. The supernatant was evaporated overnight and redissolved in distilled water.
- ***Extract C (Ethanol extract)*****:** The lyophilized mucus (1 mg/mL) was suspended in 95% ethanol and centrifuged at 10,000 rpm for 30 minutes. The supernatant was evaporated and re-dissolved in 95% ethanol, and the extraction was repeated three times. The pooled ethanol extract was evaporated, reconstituted in distilled water to a final volume of 50 mL, and further extracted with dichloromethane (CH_2Cl_2).
- ***Extract D (DMSO extract)*****:** The lyophilized residue obtained from Extract C was dissolved in distilled water containing 5% dimethyl sulfoxide (DMSO)

Antibacterial activity

The antibacterial activity of mucus extracts (A, B, C, and D) was evaluated against selected human pathogenic bacteria, namely *Escherichia coli, Vibrio cholerae, Staphylococcus aureus,* and fish pathogenic bacteria including *Yersinia ruckeri, Aeromonas hydrophila,* and *Pseudomonas aeruginosa* (IMTECH, Chandigarh, India). Antibacterial activity was assessed using the standard agar well diffusion method (Pickering et al, 1974). Zones of inhibition around the 3 mm diameter wells were measured in millimeters.

Antifungal activity

The antifungal activity of mucus extracts was tested against fungal strains such as *Aspergillus niger, Aspergillus flavus* and *Candida albicans*. Fungal cultures were grown on potato dextrose agar medium for 24 hours and adjusted to a final concentration of approximately 10^4 CFU/mL using McFarland standards. Five percent of each extract was applied to the inoculated media and incubated at 28 °C. Inhibition zones were measured after 24 hours of incubation. A standard antifungal agent was used as a positive control.

Statistical analysis

All experimental data were subjected to one-way analysis of variance (ANOVA) to evaluate treatment effects. Mean differences were analyzed using Duncan's Multiple Range Test (DMRT) with SPSS software (Version 13).

III. Result

Solubility

Solubility is the ability of a substance to dissolve in a solvent, depending on the chemical and physical properties of both the solute and solvent, as well as temperature, pressure, and other solution components. The solubility of *Macrognathus siamensis* epidermal mucus was evaluated in various polar and non-polar solvents (Table 1).

Table 1. Solubility of *M. siamensis* epidermal mucus in different solvents

Solvent	Solubility
Milli-Q water	Soluble
Methanol	Insoluble
Acetonitrile (ACN)	Insoluble
Chloroform	Insoluble
Trifluoroacetic acid (TFA)	Soluble

The mucus was soluble only in Milli-Q water and TFA, indicating its polar nature.

pH

The mucus exhibited a pH of **5.46**, indicating a slightly acidic nature.

Electrical Conductivity

Electrical conductivity measures a solution's ability to conduct an electric current, arising from mobile ions, and is the reciprocal of electrical resistivity. The mucus of *M. siamensis* showed a conductivity of **0.365 mS/cm**, suggesting the presence of charged ions in the sample.

Melting Point

The melting point of the mucus was recorded to assess thermal stability. The sample charred at **205 °C**, likely due to its carbohydrate content, forming a residual carbonaceous matter.

Biochemical Composition

Carbohydrate Content

Total carbohydrate content of the mucus was measured using the anthrone method. The mucus indicating a moderate presence of polysaccharides in the epidermal secretions (Fig. 1). The graph implies the concentration of carbohydrate concentration in the mucus sample with the comparative results of glucose as standard.

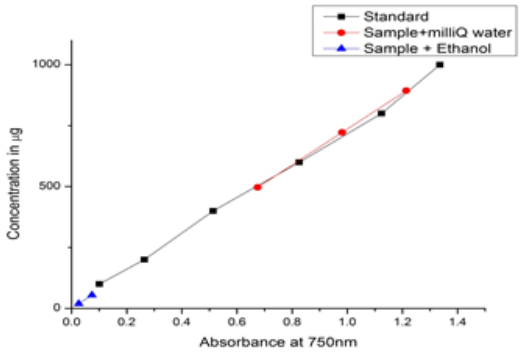

Fig. 1. Calibration curve of standard and fish mucus dissolved in milliQ water and ethanol.

Protein Content

Total protein content, estimated by the Lowry method, reflecting the mucus's role in antimicrobial defense and structural integrity (Fig. 2). The above graph implies the concentration of protein concentration in the mucus sample with the comparative results of Bovine serum albumin as standard.

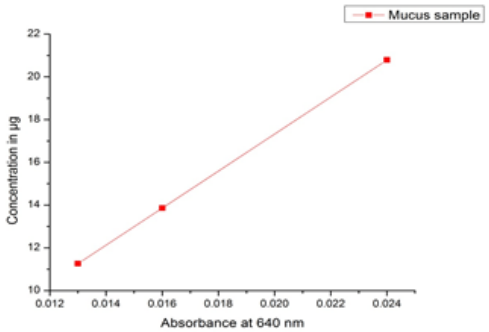

Fig. 2. Protein concentration in the fish mucus

FTIR Analysis

FTIR spectra of the powdered mucus exhibited characteristic peaks corresponding to functional groups present in proteins, carbohydrates, and lipids. Prominent absorption bands were observed at 3,400 cm^{-1} (O–H/N–H stretching), 1,650 cm^{-1} (C=O stretching of amides), 1,050 cm^{-1} (C–O stretching of carbohydrates), confirming the complex biochemical composition of the mucus (Fig. 3). The graph shows the functional group present in the mucus sample.

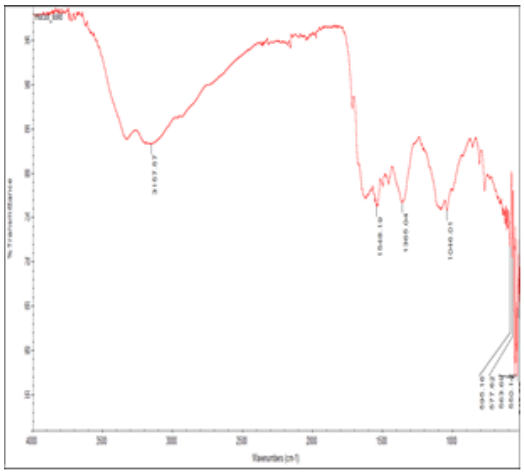

Fig. 3. FT-IR Spectra of fish mucus

HPLC Analysis

HPLC profiling of the mucus revealed distinct peaks corresponding to carbohydrates, amino acids, and small molecular metabolites. Retention times and peak areas were calibrated against standards, confirming the presence of bioactive components in the epidermal secretions. The above peak implies that the sample mucus contains biochemical compounds at the absorbance of 205nm.

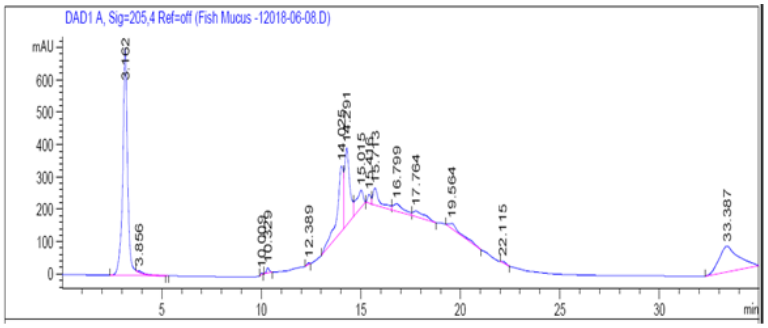

Fig. 4. HPLC Spectra of fish mucus

UV-Visible Spectrophotometry

UV-Vis spectral analysis of the mucus solution showed absorption maxima at **280 nm and 320 nm**, indicating the presence of proteinaceous compounds and conjugated molecules. These results support the biochemical composition observed in FTIR analyses.

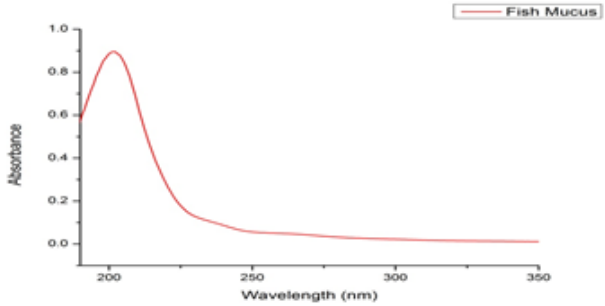

Fig. 5. UV Spectra of fish mucus

Antimicrobial Activity of Macrognathus siamensis Mucus

The antibacterial and antifungal potential of *M. siamensis* epidermal mucus was evaluated against selected human, fish, and fungal pathogens using the agar well diffusion method. The results are summarized in Tables 2- 4.

Activity Against Human Pathogens

The mucus exhibited significant antibacterial activity against all tested human pathogens (Table 2). Extract D showed the highest inhibitory effect, with zones of inhibition measuring **14.0 ± 2.0 mm** against *Escherichia coli*, **11.0 ± 2.0 mm** against *Vibrio cholerae*, and **12.0 ± 1.0 mm** against *Staphylococcus aureus*. Extract B and C also displayed moderate activity, whereas Extract A showed the least inhibition. Notably, all mucus extracts demonstrated greater antibacterial activity than the standard antibiotic Ampicillin, which produced zones of inhibition of 4–5 mm. These results indicate that *M. siamensis* mucus possesses broad-spectrum antibacterial properties, particularly against Gram-negative and Gram-positive human pathogens.

Table 2. ***Antibacterial activity of Macrognathus siamensis mucus against human pathogens***
(Zone of inhibition in mm; Mean ± SD)

Dilution (1 mg/mL)	*Escherichia coli*	*Vibrio cholerae*	*Staphylococcus aureus*
Extract A	11.00 ± 1.50^{a}	8.00 ± 2.00^{a}	7.00 ± 1.00^{b}
Extract B	13.00 ± 1.00^{a}	9.00 ± 1.50^{ab}	10.00 ± 2.00^{ab}
Extract C	12.00 ± 1.00^{a}	7.00 ± 1.00^{b}	8.00 ± 1.00^{b}
Extract D	14.00 ± 2.00^{a}	11.00 ± 2.00^{a}	12.00 ± 1.00^{a}
Ampicillin	5.00 ± 1.00^{b}	4.00 ± 1.00^{b}	5.00 ± 1.00^{c}

Mean values within a column followed by different superscripts differ significantly ($p < 0.05$).

Activity Against Fish Pathogens

The antibacterial activity of mucus against fish pathogens (*Yersinia ruckeri*, *Aeromonas hydrophila*, and *Pseudomonas aeruginosa*) is presented in Table 3. Extract D exhibited the strongest inhibitory effect, with zones of inhibition of **15.0 ± 2.0 mm** for *Y. ruckeri*, **16.0 ± 1.0 mm** for *A. hydrophila*, and **11.0 ± 2.0 mm** for *P. aeruginosa*. All mucus extracts outperformed Ampicillin (zones of inhibition 9–10 mm), indicating potent antibacterial activity relevant for aquaculture pathogens.

Table 3. ***Antibacterial activity of Macrognathus siamensis mucus against fish pathogens***
(Zone of inhibition in mm; Mean ± SD)

Dilution (1 mg/mL)	*Yersinia ruckeri*	*Aeromonas hydrophila*	*Pseudomonas aeruginosa*
Extract A	13.00 ± 1.00^{ab}	15.00 ± 2.00^{a}	12.00 ± 2.00^{a}
Extract B	12.00 ± 1.50^{ab}	14.00 ± 1.00^{a}	13.00 ± 1.00^{a}
Extract C	14.00 ± 1.00^{a}	15.00 ± 1.00^{a}	12.00 ± 1.00^{a}
Extract D	15.00 ± 2.00^{a}	16.00 ± 1.00^{a}	11.00 ± 2.00^{a}
Ampicillin	10.00 ± 1.50^{b}	9.00 ± 1.00^{b}	9.00 ± 2.00^{b}

Mean values within a column followed by different superscripts differ significantly ($p < 0.05$).

Activity Against Fungal Pathogens

The antifungal activity of mucus was assessed against *Aspergillus niger*, *Aspergillus flavus*, and *Candida albicans* (Table 4). Extract D showed the highest activity against *A. niger* (11.0 ± 1.0 mm) and *A. flavus* (10.0 ± 1.0 mm), whereas Extract B demonstrated the greatest inhibition against *C. albicans* (10.0 ± 1.0 mm). Extracts A and C also displayed moderate activity, all significantly higher than the standard control Ampicillin (4–5 mm). These findings suggest that *M. siamensis* mucus possesses broad-spectrum antifungal activity.

Table 4. ***Antifungal activity of Macrognathus siamensis mucus against fungal pathogens***
(Zone of inhibition in mm; Mean ± SD)

Dilution (1 mg/mL)	*Aspergillus niger*	*Aspergillus flavus*	*Candida albicans*
Extract A	8.00 ± 1.00^{b}	9.00 ± 1.00^{a}	9.00 ± 2.00^{ab}
Extract B	7.00 ± 1.00^{b}	7.00 ± 1.00^{b}	10.00 ± 1.00^{a}
Extract C	8.00 ± 1.50^{b}	9.00 ± 1.00^{a}	8.00 ± 1.00^{ab}
Extract D	11.00 ± 1.00^{a}	10.00 ± 1.00^{a}	8.00 ± 2.00^{ab}
Ampicillin	4.00 ± 1.00^{c}	5.00 ± 1.00^{c}	5.00 ± 1.00^{b}

Mean values within a column followed by different superscripts differ significantly ($p < 0.05$).

IV. Discussion

The present study demonstrates that the epidermal mucus of *Macrognathus siamensis* possesses distinct physicochemical, biochemical, and antimicrobial properties, supporting its potential as a source of bioactive compounds.

Physicochemical Properties

Solubility analysis revealed that the mucus was soluble only in Milli-Q water and trifluoroacetic acid (TFA), indicating its polar nature. Similar observations were reported in *Macrognathus armatus* and other spiny eels, where mucus exhibited solubility primarily in polar solvents due to its proteinaceous and polysaccharide components (Vankara et al, 2011).

The pH of the mucus (5.46) was slightly acidic, which is consistent with previous reports in freshwater fishes, where epidermal secretions maintain a mildly acidic environment to inhibit microbial colonization (Subramanian et al, 2007). Such acidity may contribute to the antimicrobial potential of the mucus.

The electrical conductivity of 0.365 mS/cm indicates the presence of dissolved ions, which could be associated with small metabolites, salts, and charged proteins in the mucus matrix. This observation aligns with previous studies showing that fish mucus contains electrolytes that support ion transport and osmotic balance (Esteban, 2012).

The melting point analysis revealed charring at 205 °C, likely due to carbohydrate content. Similar thermal properties have been observed in mucus samples of other teleost fishes, suggesting that polysaccharides and glycoproteins contribute to structural stability and resistance to heat-induced degradation (Matsui et al, 2000).

Biochemical Composition

The mucus of *M. siamensis* contained carbohydrates, proteins, and lipids, confirming its multifunctional nature. Polysaccharides and glycoproteins play roles in lubrication, protection, and antimicrobial defense (Subramanian et al, 2008). Proteins in mucus are known to include antimicrobial peptides, lysozymes, and lectins, which inhibit microbial growth and provide structural integrity (Esteban, 2012).

FTIR and HPLC analyses further corroborated the presence of functional groups and bioactive molecules. Peaks corresponding to O–H, N–H, and C=O groups reflect proteins and carbohydrates, while HPLC confirmed small metabolites, amino acids, and other bioactive compounds. UV-Vis absorption at 280–320 nm indicates aromatic amino acids and conjugated molecules, supporting the presence of antimicrobial peptides and glycoproteins (Ross et al, 2010).

Antimicrobial Activity

The mucus of *M. siamensis* exhibited broad-spectrum antimicrobial activity. Against human pathogens, Extract D displayed the strongest activity, surpassing the standard antibiotic Ampicillin in inhibition zones. The inhibitory effect against Gram-negative (*E. coli*, *V. cholerae*) and Gram-positive (*S. aureus*) bacteria suggests the presence of potent antimicrobial peptides and bioactive metabolites, consistent with previous reports in freshwater fish mucus (Subaramanian et al, 2008).

Similarly, the mucus showed strong antibacterial activity against aquaculture-relevant pathogens (*Y. ruckeri*, *A. hydrophila*, *P. aeruginosa*), highlighting its potential as a natural biocontrol agent. Previous studies have demonstrated that fish mucus contains lectins, lysozymes, and immunoglobulin-like proteins that confer resistance to fish pathogens (Esteban, 2012).

The antifungal activity against *Aspergillus niger*, *A. flavus*, and *Candida albicans* indicates that mucus compounds are effective beyond bacteria. Extract D and B exhibited the highest inhibitory activity, suggesting a variation in bioactive components among mucus samples. Similar antifungal effects have been observed in epidermal mucus of *Mastacembelus armatus* and other teleosts, attributed to glycoproteins and antimicrobial peptides (Shephard, 1994).

V. Conclusion

The epidermal mucus of *Macrognathus siamensis* exhibits a polar nature, slightly acidic pH, and contains carbohydrates, proteins, and lipids, contributing to its structural and defensive functions. It demonstrates broad-spectrum antibacterial and antifungal activity against human, fish, and fungal pathogens, highlighting its role in innate immunity. These findings suggest that *M. siamensis* mucus is a promising source of bioactive compounds with potential applications in aquaculture disease management and the development of natural antimicrobial agents.

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