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ANTIBACTERIAL POTENTIAL OF MANGOBAI FISH MUCUSN (*Stiphodon sp.*) EXTRACTED WITH DIFFERENT CONCENTRATIONS OF ACETIC ACID SOLVENT

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ABSTRACT
Fish skin mucus contains various bioactive compounds, including antimicrobial peptides, which have potential as natural antibacterial agents. This study aimed to evaluate the antibacterial activity of Mangobai fish (*Stiphodon* sp.) mucus extracted using different concentrations of acetic acid (3%, 5%, and 7%) against *Staphylococcus aureus* and *Escherichia coli*. A laboratory experimental design was employed using the Kirby–Bauer disk diffusion method. Chloramphenicol and sterile distilled water served as positive and negative controls, respectively. Inhibition zone diameters were measured after 24 h of incubation. Data were analyzed using the Kolmogorov–Smirnov and Shapiro–Wilk normality tests, followed by the Kruskal–Wallis test and Duncan's Multiple Range Test. All mucus extracts exhibited antibacterial activity against both bacterial species. The 7% acetic acid extract produced the largest inhibition zones among the extract treatments, measuring 11.976 mm against *S. aureus* and 10.700 mm against *E. coli*. The Kruskal–Wallis test showed significant differences among treatments for both *E. coli* ($p = 0.001$) and *S. aureus* ($p < 0.001$). Duncan's test indicated that the 3% extract provided antibacterial activity statistically comparable to higher concentrations against *E. coli*, whereas the 5% extract showed activity comparable to the 7% extract against *S. aureus*. *Stiphodon* sp. mucus possesses promising antibacterial activity, and acetic acid concentration significantly influences extraction efficiency. The 3% acetic acid extract was the most efficient concentration for *E. coli*, whereas the 5% extract was optimal for *S. aureus*.

INTRODUCTION

Antimicrobial resistance (AMR) has become one of the most critical global public health challenges, reducing the effectiveness of existing antibiotics and increasing the prevalence of difficult-to-treat bacterial infections. The excessive and inappropriate use of antibiotics in human medicine, veterinary practice, and agriculture has accelerated the emergence of multidrug-resistant microorganisms, creating an urgent need to discover alternative antimicrobial agents from natural resources [1][2][3]. Consequently, increasing attention has been directed toward bioactive compounds derived from aquatic organisms as sustainable sources of novel antibacterial substances.

Fish skin mucus functions as the first biological defense barrier against pathogenic microorganisms in aquatic environments. It contains numerous bioactive compounds, including antimicrobial peptides, glycoproteins, lysozymes, lectins, proteases, and low-molecular-weight proteins that exhibit broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria [4][5]. Recent studies have demonstrated that epidermal mucus from various freshwater fish species possesses considerable antibacterial

activity, highlighting its potential as a promising natural alternative for combating antimicrobial resistance [6]. Nevertheless, the antibacterial properties of many indigenous freshwater fish species remain poorly explored.

One freshwater fish with considerable ecological and economic importance is the Mangobai fish (*Stiphodon* sp.), a member of the Gobiidae family naturally inhabiting Lake Limboto, Gorontalo, Indonesia. Lake Limboto is the largest freshwater lake in Gorontalo Province and plays essential ecological, economic, and social roles for surrounding communities. Besides functioning as an important inland fishery, the lake supports local livelihoods through fisheries and aquaculture activities. Among its native fish resources, Mangobai fish is highly valued because of its commercial value and consumer preference. Although the species has traditionally been utilized as food, its biological resources, particularly skin mucus, have received limited scientific attention despite their potential as a source of natural antimicrobial compounds.

Fish mucus has attracted growing scientific interest because it naturally protects fish against microbial invasion through the secretion of antimicrobial peptides and immune-related proteins. The successful recovery of these bioactive compounds depends greatly on the extraction method employed. Acidic solvents, particularly acetic acid, are widely recognized as effective extraction media because they improve the solubility of cationic antimicrobial peptides while preserving their biological activity [5]. Previous investigations have shown that acidic extraction generally produces stronger antibacterial activity than neutral solvents, suggesting that extraction conditions strongly influence the antimicrobial effectiveness of fish mucus [6][7].

Despite these promising findings, information regarding the optimal concentration of acetic acid for extracting antibacterial compounds from *Stiphodon* sp. mucus remains limited. Previous local research reported antibacterial activity of Mangobai fish mucus extracted using 3% acetic acid, whereas the influence of higher solvent concentrations has not been comprehensively evaluated. Since acetic acid concentration affects extraction efficiency, peptide solubility, and recovery of bioactive compounds, determining the optimal solvent concentration is essential for maximizing antibacterial activity. Furthermore, most published studies have focused on commonly investigated fish species, leaving *Stiphodon* sp. largely unexplored despite its local abundance and economic importance.

The antibacterial activity of fish mucus is particularly relevant against clinically important pathogens such as *Staphylococcus aureus* and *Escherichia coli*. *Staphylococcus aureus* is a major Gram-positive bacterium responsible for skin infections, wound infections, pneumonia, and endocarditis, whereas *Escherichia coli*, a Gram-negative bacterium, is one of the leading causes of gastrointestinal infections and diarrhea. The increasing resistance of these bacteria to conventional antibiotics highlights the importance of identifying novel natural antibacterial agents capable of inhibiting their growth [2].

Therefore, this study aimed to evaluate the antibacterial potential of Mangobai fish (*Stiphodon* sp.) mucus extracted using different concentrations of acetic acid (3%, 5%, and 7%) against *Staphylococcus aureus* and *Escherichia coli*. The novelty of this study lies in the

comparative evaluation of different acetic acid concentrations for extracting antibacterial compounds from the mucus of an indigenous freshwater fish species from Lake Limboto. The findings are expected to contribute to the development of natural antibacterial agents derived from local aquatic biodiversity while providing scientific evidence for optimizing fish mucus extraction methods to support future pharmaceutical and biomedical applications.

Literatur Review

Fish Skin Mucus as a Natural Source of Antibacterial Compounds

Fish skin mucus is an essential component of the innate immune system that protects fish from pathogenic microorganisms in aquatic environments. As the first biological barrier against bacterial invasion, mucus contains a complex mixture of bioactive substances, including antimicrobial peptides (AMPs), lysozyme, lectins, immunoglobulins, glycoproteins, enzymes, proteases, and other low-molecular-weight compounds that collectively inhibit microbial colonization [4][5]. These compounds function through both physical and biochemical defense mechanisms, allowing fish to survive in environments with high microbial loads.

Recent studies have highlighted fish skin mucus as a promising natural source of antibacterial agents because of its broad-spectrum activity against Gram-positive and Gram-negative bacteria. According to Díaz-Puertas et al. [4], fish mucus remains an underexplored reservoir of antimicrobial compounds with considerable potential for pharmaceutical and biomedical applications. Likewise, Lee et al. [5] demonstrated that mucus extracted from several freshwater and marine fish species effectively inhibited bacterial growth, suggesting its potential as an alternative to conventional antibiotics.

Experimental evidence also indicates that crude mucus extracts from freshwater fish exhibit significant inhibitory activity against pathogenic bacteria. Ranjini et al. [8], reported that skin mucus obtained from *Catla catla* and *Channa striatus* produced clear inhibition zones against *Staphylococcus aureus* and *Escherichia coli*. Similar antibacterial properties have been observed in *Hypophthalmichthys nobilis* and several carp species, supporting the hypothesis that fish mucus possesses naturally occurring antimicrobial compounds capable of suppressing pathogenic microorganisms [9][10].

Despite the increasing body of evidence regarding antibacterial properties of fish mucus, studies involving Mangobai fish (*Stiphodon* sp.) remain extremely limited. Most available investigations have focused on commercially important freshwater or marine fish species, leaving *Stiphodon* spp. largely unexplored. This knowledge gap provides a strong rationale for investigating the antibacterial potential of Mangobai fish mucus as a novel natural antimicrobial source.

Antimicrobial Peptides in Fish Skin Mucus

The antibacterial activity of fish mucus is primarily attributed to antimicrobial peptides (AMPs), which are evolutionarily conserved molecules constituting a major component of innate immunity. AMPs exhibit broad-spectrum antimicrobial activity against bacteria, fungi, parasites, and viruses while minimizing the likelihood of microbial resistance because they target fundamental cellular structures rather than specific metabolic pathways [4].

Fish-derived AMPs generally possess amphipathic and cationic characteristics that enable them to interact strongly with negatively charged bacterial membranes. Following attachment, these peptides disrupt membrane integrity, resulting in pore formation, leakage of intracellular contents, osmotic imbalance, and ultimately bacterial cell death [11].

Okella et al. [11], successfully isolated several antimicrobial peptides from the skin mucus of African catfish (*Clarias gariepinus*) and demonstrated potent antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. Likewise, Anju et al. [12], identified crude peptide extracts from *Arius jella* that effectively inhibited Gram-positive and Gram-negative bacteria, suggesting that fish-derived AMPs possess broad-spectrum antibacterial properties.

Beyond membrane disruption, AMPs may interfere with bacterial DNA replication, RNA transcription, protein synthesis, and intracellular metabolic pathways, thereby providing multiple mechanisms of antibacterial action [11]. These diverse mechanisms make AMPs attractive candidates for developing alternative antimicrobial agents in response to the growing problem of antimicrobial resistance.

Acetic Acid Extraction of Fish Mucus Bioactive Compounds

The effectiveness of fish mucus as an antibacterial agent depends largely on the extraction method used to isolate its bioactive compounds. Acidic solvents, particularly acetic acid, are among the most widely applied extraction media because they efficiently solubilize antimicrobial peptides and low-molecular-weight proteins while preserving their biological activity [5].

Lee et al. [5], compared various extraction solvents and concluded that acidic extraction generally produces greater antibacterial activity than aqueous extraction methods. Acidic conditions facilitate the release of cationic peptides responsible for antibacterial activity, resulting in extracts with enhanced inhibitory capacity.

Optimization studies have also demonstrated that extraction conditions substantially influence peptide yield and antibacterial effectiveness. Wang et al. [13], reported that optimizing acetic acid extraction conditions significantly increased recovery of antibacterial proteins from fish by-products. Similarly, Zhang et al. [14], found that ultrasound-assisted acetic acid extraction improved peptide recovery and produced extracts with stronger antibacterial activity against pathogenic bacteria.

Although acetic acid has become the preferred solvent for antimicrobial peptide extraction, few studies have systematically evaluated the influence of different acetic acid concentrations on antibacterial activity. Consequently, determining the optimal solvent concentration remains an important research objective, particularly for underexplored fish species such as *Stiphodon* sp.

Antibacterial Mechanisms Against *Staphylococcus aureus* and *Escherichia coli*

Staphylococcus aureus and *Escherichia coli* are commonly used model bacteria in antibacterial studies because they represent Gram-positive and Gram-negative pathogens responsible for numerous human infections.

Staphylococcus aureus is an opportunistic pathogen associated with skin infections, pneumonia, septicemia, wound infections, osteomyelitis, and infective endocarditis. Its increasing resistance to antibiotics has become a

major global health concern. Conversely, *Escherichia coli* is a normal intestinal bacterium, although pathogenic strains can cause diarrhea, urinary tract infections, septicemia, and gastrointestinal diseases.

Natural antibacterial compounds isolated from fish mucus inhibit these bacteria through several complementary mechanisms. The primary mechanism involves disruption of bacterial cell membranes, resulting in leakage of intracellular proteins, nucleic acids, potassium ions, and other essential cellular constituents, ultimately causing cell lysis [15].

In addition to membrane disruption, antibacterial compounds may inhibit DNA replication and protein synthesis, preventing bacterial growth and reproduction. Some antimicrobial compounds also induce oxidative stress by stimulating excessive production of reactive oxygen species (ROS), leading to irreversible damage to bacterial membranes, proteins, and genetic material [16].

Because these mechanisms target multiple cellular structures simultaneously, fish mucus-derived antimicrobial peptides are considered promising alternatives to conventional antibiotics, particularly against multidrug-resistant bacteria.

RESEARCH METHODS

Research Design

This study employed a quantitative laboratory experimental design to evaluate the antibacterial activity of Mangobai fish (*Stiphodon* sp.) skin mucus extracted using different concentrations of acetic acid [17]. The antibacterial effects of the extracts were tested against Staphylococcus aureus and Escherichia coli using the Kirby–Bauer disk diffusion method. The experiment consisted of four treatment groups, namely 3% acetic acid extract, 5% acetic acid extract, 7% acetic acid extract, a positive control (chloramphenicol), and a negative control, with five replications for each treatment.

Collection of Fish Skin Mucus

Mangobai fish (*Stiphodon* sp.) were collected from Lake Limboto, Gorontalo, Indonesia. Healthy fish were placed individually into sterile polyethylene bags and gently shaken for approximately 10–20 minutes to stimulate epidermal mucus secretion without causing physical injury. The collected mucus was transferred into sterile glass beakers, labeled, and stored at 4°C until extraction.

Preparation of Fish Mucus Extract

The mucus was extracted using 3%, 5%, and 7% (v/v) acetic acid solutions. Each extract was prepared by mixing fish mucus with the corresponding acetic acid solution in a 1:1 ratio. For example, 10 mL of fish mucus was mixed with 10 mL of acetic acid solution.

The mixture was heated in a water bath for 5 minutes, cooled on ice, and centrifuged at 9,000 rpm for 45 minutes at 10°C. The resulting supernatant was collected aseptically using sterile micropipettes and stored at 4°C until antibacterial testing.

Preparation of Bacterial Cultures

Pure cultures of Staphylococcus aureus and Escherichia coli were used as test microorganisms.

Nutrient Broth (NB) medium was prepared by dissolving 0.2 g of NB powder in 25 mL of distilled water and sterilized at 121°C for 15 minutes. Each bacterial isolate was inoculated into sterile NB medium and incubated in a shaking incubator at 37°C and 160 rpm for 48 hours.

The turbidity of each bacterial suspension was adjusted using a spectrophotometer at 580 nm until an optical density (OD) of 0.6 was obtained before antibacterial testing.

Antibacterial Activity Assay

The antibacterial activity of Mangobai fish mucus extracts was evaluated using the Kirby–Bauer disk diffusion method.

One milliliter of the standardized bacterial suspension was transferred into sterile Petri dishes, followed by the addition of 30 mL of sterile Nutrient Agar (NA) at approximately 45°C using the pour plate technique.

Sterile paper discs were immersed in each fish mucus extract for 30 minutes before being placed on the inoculated agar surface. Chloramphenicol discs served as the positive control, whereas sterile discs without fish mucus extract served as the negative control.

All culture plates were incubated at 37°C for 24 hours. Following incubation, antibacterial activity was determined by measuring the diameter of the inhibition zone surrounding each disc using a digital Vernier caliper. Measurements were recorded in millimeters (mm).

Data Analysis

The inhibition zone diameters obtained from each treatment were expressed as mean values from five replications.

Data normality was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. Because several treatment groups showed significance values of $p < 0.05$, the data were considered not normally distributed. Therefore, statistical analysis was continued using the non-parametric Kruskal–Wallis test to determine differences in antibacterial activity among treatment groups.

When the Kruskal–Wallis test indicated statistically significant differences ($p < 0.05$), a Duncan multiple range test was performed as a post hoc analysis to identify differences among treatment groups and determine the optimum acetic acid concentration for antibacterial activity.

All statistical analyses were conducted using IBM SPSS Statistics, with statistical significance established at $\alpha = 0.05$.

RESEARCH RESULT

Antibacterial Activity of *Stiphodon* sp. Mucus Extract

The antibacterial activity of *Stiphodon* sp. (Mangobai fish) mucus extract was evaluated against *Staphylococcus aureus* and *Escherichia coli* using the Kirby–Bauer disk diffusion method. The extract was prepared using three concentrations of acetic acid solvent (3%, 5%, and 7%). Chloramphenicol served as the positive control, while sterile distilled water served as the negative control. The diameter of the inhibition zone was measured using a digital caliper after 24 h of incubation.

Table 1. Mean inhibition zone diameter of *Stiphodon* sp. mucus extract against *Staphylococcus aureus* and *Escherichia coli*

Treatment	S. aureus (mm)	Category	E. coli (mm)	Category
Negative control	0.000	No inhibition	0.000	No inhibition
3% acetic acid	7.602	Moderate	7.230	Moderate
5% acetic acid	8.820	Moderate	9.502	Moderate
7% acetic acid	11.976	Strong	10.700	Strong

Positive control	25.592	Very Strong	26.244	Very Strong

Table 1 demonstrates that Mangobai fish mucus extract inhibited the growth of both S. aureus and E. coli at all acetic acid concentrations tested. The antibacterial activity increased as the concentration of acetic acid increased. The 7% acetic acid extract produced the largest inhibition zones among the extract treatments, measuring 11.976 mm against S. aureus and 10.700 mm against E. coli, both classified as strong antibacterial activity. As expected, chloramphenicol exhibited the highest inhibition against both bacterial species, whereas no inhibition zone was observed in the negative control.

The inhibition zones produced by each treatment are illustrated in Figure 1.

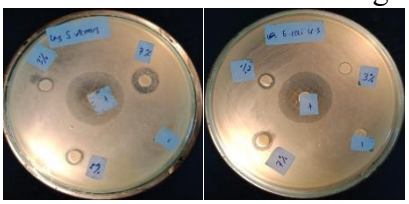

Figure 1. Inhibition zones produced by Stiphodon sp. mucus extract extracted with different acetic acid concentrations against (A) *Staphylococcus aureus* and (B) *Escherichia coli*.

Statistical Analysis

Normality Test

Prior to hypothesis testing, data normality was assessed using the Kolmogorov–Smirnov and Shapiro–Wilk tests. The results are presented in Table 2.

Table 2. Normality test of inhibition zone diameter

	Test of Normality						
		Kolmogorov-Smirnova			Shapiro-Wilk		
	Concentration	Statistics	df	Sig	Statistics	df	Sig
Inhibitory Power of Escherichia coli Bacteria	Negative Control	-	5	-	-	5	-
	Concentration 3%	188	5	200	907	5	452
	Concentration 5%	367	5	027	781	5	056
	Concentration 7%	397	5	010	697	5	009
	Positive Control	374	5	021	796	5	075
Inhibitory Power of Staphylococcus aureus Bacteria	Negative Control	-	5	-	-	5	-
	Concentration 3%	415	5	005	664	5	004
	Concentration 5%	452	5	001	608	5	001
	Concentration 7%	189	5	200	959	5	804
	Positive Control	370	5	024	691	5	008

Based on the results shown in Table 2, several treatment groups had significance values below 0.05 in either the Kolmogorov–Smirnov or Shapiro–Wilk tests, indicating that the inhibition zone data were not normally distributed. Therefore, non-parametric statistical analysis was employed using the Kruskal–Wallis test.

Kruskal–Wallis Test

The Kruskal–Wallis test was performed to determine whether different acetic acid concentrations significantly affected the antibacterial activity of Mangobai fish mucus extract.

Table 3. Kruskal–Wallis test results

Variable	H	df	p-value
E. coli	19.967	4	0.001
S. aureus	21.038	4	<0.001

The Kruskal–Wallis analysis revealed statistically significant

differences among treatment groups for both bacterial species. The inhibition zones against E. coli differed significantly among treatments ($H = 19.967$, $p = 0.001$), while those against S. aureus also differed significantly ($H = 21.038$, $p < 0.001$). These findings indicate that different concentrations of acetic acid used during extraction significantly influenced the antibacterial activity of Stiphodon sp. mucus extract.

Post Hoc Analysis

To identify which treatment groups differed significantly, Duncan's Multiple Range Test was conducted.

Escherichia coli

Table 4. Duncan's multiple range test for *Escherichia coli*

Inhibitory Power of E.coli Bacteria				
Duncan				
Subtet for alpha = 0.05				
Concentration	N	1	2	3
Negative Control	5	0000		
Concentration 3%	5		7.2300	
Concentration 5%	5		9.5020	
Concentration 7%	5		10.7000	
Positive Control	5			26.2440
Sig		1.000	007	1.000

The Duncan test showed that the negative control differed significantly from all extract treatments and the positive control. However, the inhibition zones produced by the 3%, 5%, and 7% acetic acid extracts were grouped within the same subset, indicating no statistically significant differences among these three concentrations ($p > 0.05$). Consequently, although the 7% extract produced the largest inhibition zone numerically, the 3% extract may be considered the most efficient concentration because it achieved comparable antibacterial activity using a lower concentration of acetic acid.

Staphylococcus aureus

Table 5. Duncan's multiple range test for *Staphylococcus aureus*

Inhibitory Power of Staphylococcus aureus Bacteria					
Duncan					
Subtet for alpha = 0.05					
Concentration	N	1	2	3	4
Negative Control	5	0000			
Concentration 3%	5		7.6040		
Concentration 5%	5		9.8200	8.8200	
Concentration 7%	5			11.7760	
Positive Control	5				25.5920
Sig		1.000	471	089	1.000

The Duncan test demonstrated significant differences between the negative control and the treated groups. However, the inhibition zones produced by the 5% and 7% acetic acid extracts belonged to overlapping subsets, indicating no significant difference between these two concentrations. Although the 7% extract generated the largest inhibition zone numerically, the 5% extract can be regarded as the optimum concentration because it produced statistically comparable antibacterial activity while requiring a lower concentration of acetic acid.

DISCUSSION

Antibacterial Activity of *Stiphodon* sp. Mucus Extract

The present study demonstrated that mucus extracted from *Stiphodon* sp. exhibited antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. All extracts prepared with acetic acid (3%, 5%, and 7%) produced measurable inhibition zones, whereas the negative control showed no

antibacterial effect. Furthermore, increasing the concentration of acetic acid during extraction generally increased the inhibition zone diameter, with the 7% acetic acid extract producing the strongest antibacterial activity among the tested extracts. These findings indicate that the extraction solvent concentration substantially influences the recovery of antibacterial compounds from fish mucus.

Fish skin mucus is recognized as an essential component of the innate immune system of fish and serves as the first protective barrier against microbial invasion. It contains numerous bioactive molecules, including antimicrobial peptides (AMPs), glycoproteins, lectins, lysozymes, proteases, complement proteins, and immunoglobulins, all of which contribute to antimicrobial defense [4][5]. The antibacterial activity observed in the present study therefore likely reflects the combined effects of these bioactive compounds rather than a single antimicrobial component.

These findings are consistent with previous reports showing that fish mucus possesses broad-spectrum antibacterial activity against both Gram-positive and Gram-negative bacteria. Anju et al. [12], demonstrated that crude peptide extracts isolated from the skin mucus of *Arius jella* effectively inhibited *S. aureus* through the activity of naturally occurring antimicrobial peptides. Similarly, Lee et al. [5], reported significant antibacterial activity in epidermal mucus protein extracts from freshwater fish, confirming that fish mucus constitutes a promising source of natural antibacterial agents. Comprehensive reviews by Díaz-Puertas et al. [4] and Tiralongo et al. [18] further emphasized that fish mucus represents an underexplored reservoir of antimicrobial compounds with considerable pharmaceutical potential.

Effect of Acetic Acid Concentration on Antibacterial Activity

The increase in antibacterial activity observed with increasing acetic acid concentration can be explained by the extraction efficiency of antimicrobial peptides and other low-molecular-weight proteins. Acidic extraction has long been recognized as one of the most effective methods for recovering cationic antimicrobial peptides because slightly acidic conditions improve peptide solubility while reducing enzymatic degradation during extraction [5].

Acetic acid disrupts interactions between proteins and mucus glycoproteins, facilitating the release of bioactive peptides into solution. Consequently, higher concentrations of acetic acid generally extract greater amounts of antibacterial compounds, resulting in larger inhibition zones. Similar observations have been reported in peptide extraction studies where acidic extraction produced significantly higher antibacterial activity than aqueous extraction methods [5].

The present results agree with optimization studies conducted by Wang et al. [13], who demonstrated that acetic acid extraction effectively recovered antibacterial proteins and peptides from fish by-products. Likewise, Kurniatin et al. [19], reported that acetic acid significantly improved antimicrobial peptide extraction efficiency from natural biological materials. Collectively, these studies support the conclusion that solvent concentration is a critical factor influencing antibacterial potency.

Interestingly, although the 7% extract produced the largest inhibition zones numerically, post hoc analysis indicated no statistically significant difference between the 3%, 5%, and 7% treatments against *E. coli*, and between the

5% and 7% treatments against *S. aureus*. This finding suggests that extraction efficiency may reach a plateau after a certain solvent concentration, where additional acetic acid no longer substantially increases the amount of active compounds recovered. Therefore, lower solvent concentrations may provide comparable antibacterial efficacy while reducing solvent consumption and processing costs.

Differences in Activity Against *Staphylococcus aureus* and *Escherichia coli*

The inhibition zones produced against *S. aureus* were consistently slightly larger than those against *E. coli*. This difference is likely associated with structural differences between Gram-positive and Gram-negative bacteria.

Gram-positive bacteria such as *S. aureus* possess a thick peptidoglycan layer but lack an outer membrane, allowing antimicrobial peptides to directly interact with the cytoplasmic membrane. In contrast, Gram-negative bacteria such as *E. coli* possess an additional outer membrane rich in lipopolysaccharides that functions as an effective permeability barrier against many antimicrobial compounds.

Numerous studies have demonstrated that antimicrobial peptides primarily exert their activity through electrostatic interactions with negatively charged bacterial membranes. These interactions promote membrane destabilization, pore formation, leakage of intracellular components, membrane depolarization, and ultimately bacterial cell death [15]. Additional mechanisms include inhibition of DNA replication, interference with protein synthesis, disruption of intracellular metabolism, and induction of oxidative stress through reactive oxygen species (ROS) generation [20].

Therefore, the slightly greater susceptibility of *S. aureus* observed in this study is biologically reasonable because Gram-positive bacteria generally present fewer structural barriers to peptide penetration than Gram-negative bacteria.

Statistical Analysis and Biological Interpretation

The Kruskal–Wallis analysis confirmed that acetic acid concentration significantly influenced the antibacterial activity of *Stiphodon* sp. mucus against both bacterial species (*E. coli*: $p = 0.001$; *S. aureus*: $p < 0.001$). These statistical findings indicate that the observed differences among treatments were unlikely to occur by chance.

Although the 7% extract produced the highest inhibition zone diameter, Duncan's Multiple Range Test revealed that the antibacterial activities of the 3%, 5%, and 7% extracts were statistically comparable against *E. coli*. Likewise, the activities of the 5% and 7% extracts against *S. aureus* were not significantly different. Consequently, from an extraction efficiency perspective, 3% acetic acid may be considered sufficient for *E. coli*, whereas 5% acetic acid appears optimal for *S. aureus*. Selecting lower solvent concentrations while maintaining equivalent antibacterial performance offers practical advantages by reducing chemical usage, lowering production costs, and minimizing environmental impact.

CONCLUSION

This study demonstrated that mucus extract from Mangobai fish (*Stiphodon* sp.) possesses antibacterial activity against both *Staphylococcus aureus* and *Escherichia coli*. The antibacterial effectiveness of the extract

was influenced by the concentration of acetic acid used during the extraction process, indicating that extraction conditions play an important role in recovering bioactive antibacterial compounds. Although higher acetic acid concentrations tended to produce greater antibacterial activity, lower concentrations were able to achieve statistically comparable effects against the tested bacteria, indicating a more efficient extraction approach. These findings suggest that *Stiphodon* sp. mucus represents a promising natural source of antibacterial compounds with potential applications in the development of alternative antimicrobial agents. Further studies are recommended to isolate and characterize the active antibacterial compounds, elucidate their mechanisms of action, and evaluate their efficacy and safety through in vivo studies and pharmaceutical formulation development.

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