

Epidermal Antimicrobial Peptides in Fish and Frogs: From Natural Defense to Biomedical Applications

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SUMMARY

The primary surface of contact between fish and their surroundings, fish mucus layers serve crucial living and conservation roles. There is a growing trend in mucus-based research in fish, and the advancement of high-throughput methods that enable the synchronized analysis of several genes and other small molecules has led to better insight into the configuration and roles of mucus. Fish mucus is a key defense against illnesses in fish. Because of its potential in aquaculture and human health, research has largely concentrated on studying the bioactive compounds (such as immune-related chemicals and antimicrobial peptides) and associated microbiota. However, external fish mucus surfaces also have a significant impact on interspecific interactions like symbiosis, parasite-host interactions, and prey-predator relationships, as well as societal roles such as shoaling, reproductive synchronization, finding appropriate habitat, and alarm signals. Because of their delicate nature, amphibians have also been used as a substitute protein source all over the world. The skins of edible amphibians, especially large salamanders and frogs, are often discarded without being used again. Nonetheless, protein and bioactive peptides (BPs) can be extracted from these wastes. Numerous biological actions, including antidiabetic, antioxidant, and antibacterial properties, have been found for a variety of BPs. Death, morbidity, and healthcare expenses are all rising as a result of antibiotic resistance. Consequently, it is imperative to create new classes of antibiotics. A relatively new class of possible antibiotics, antimicrobial peptides have broad-spectrum efficacy, fast-acting properties, and the ability to evade many of the mechanisms of drug resistance that are currently understood. The numerous BPs that have been isolated and identified from various amphibian skins or skin secretions, as well as their biological activity, are thoroughly discussed in this paper.

Keywords: Frog, Fish, Antimicrobial resistance, bioactive peptides

Citation: Sohail, M., M. A. Kamran, S. Mansha, A. Nawaz, and F. Bashir. 2025. Epidermal Antimicrobial Peptides in Fish and Frogs: From Natural Defense to Biomedical Applications Journal of Wildlife and Ecology. 9: 333-351.

Received 25 February, 2025

Revised 29 May, 2025

Accepted 01 August, 2025

Published 10 September, 2025

INTRODUCTION

Human civilization has been influenced by natural chemicals since our earliest ancestors started using them to enhance and enrich their own lives (Onodera and

Fujii, 2023). Animals are the primary source of these substances. According to (Gnanaolivu et al., 2022), animals are indeed powerful tools for healing and have long been used in people's religious, magical, and healing activities. Since a variety of pathogenic and non-pathogenic microbes interact with all living things, including fish, they have sophisticated defense mechanisms that help them survive. According to (Schultze and Aschenbrenner, 2021), the innate immune system is one mechanism that fights infections from the initial point of contact. Particular immunity, such as antibody and cell-mediated responses, is much less varied than higher (Chalmers et al., 2022). A major hazard to public health is the rise in the number of harmful microbe strains that have become resistant to several once-effective antibiotics in recent years. There is an urgent need for entirely new kinds of antimicrobial drugs because some nosocomial bacteria are already resistant to every antibiotic that is currently on the market, making them almost incurable (Tarin-Pello et al., 2022). Fish and amphibian population declines around the world are getting more and more concerning. This reduction has been linked to several infections, habitat degradation, the introduction of predators, and direct exposure to toxins and xenobiotics. The first line of defense against these diseases is the skin, and the granular glands on the skin of many, but not all, frog species produce antimicrobial compounds, which are a crucial defense mechanism. According to (D'Andrea and Romanelli, 2023), frogs can produce and release a variety of peptides that vary in their potency and specificity against the wide variety of harmful microbes found in the environment.

Fish face numerous challenges in their harsh environment. Microorganisms significantly impact fish health. They produce mucus on the dermal layer to help them escape such an environment. Environmental agitations such as hyperosmolarity and acidity, as well as microbial contamination, have been shown to alter the mucus conformation and percentage of secretion (Ordóñez-Grande et al., 2021). A variety of processes are carried out by the mucus substance released from the fish's surface, including breathing, management, motility, etc (Saxena and Preservation). Under typical circumstances, the fish may maintain a healthy system even if they are in close contact with large concentrations of pathogens in their surroundings. This may be explained by their own intricate system of natural defenses, especially those derived from broad-spectrum antibacterial compounds. Mucus compounds have been shown by numerous investigators to be very resistant to invasive diseases (Hussain et al., 2023b). The initial physical barrier that prevents microorganisms from entering fish is the mucus of the fish. With its enzymes and antibodies, it functions as a chemical barrier that can destroy invasive pathogens (Ramírez-Larrosa and Eckhard, 2022). Arachidonic acids, which were thought to be actively involved in starting tissue wound repair, were found to be abnormally abundant in the meat of *Channa striatus*, but eicosapentaenoic acids were nearly absent (Dao, 2022). Numerous fish species have shown antimicrobial action in their mucus (Díaz-Puertas et al., 2023); however, depending on the species, this activity may be exclusive to a particular bacterium (Baquero et al., 2021).

The research that are available when we evaluate the literature on freshwater fishes is primarily on coldwater fishes. *Etheostoma crossopteron*, *Cyprinus carpio*, and *C. striatus* have all been studied. Studies on the microbicidal properties of fish mucus exist; however, they only address bacteria, except for one study that looked at

fungi (Díaz-Puertas et al., 2023). *Channa punctatus* were shown to exhibit antimicrobial action (Hussain et al., 2023a). (Leonora et al., 2025) examined the antimicrobial properties of the intestinal mucus and skin of five distinct freshwater fish species, *Channa*. There is currently no research on the mucus of freshwater fish that can be cultivated aside from these. Every year, the world's fisheries discard about 20 million metric tons of fish byproducts. Numerous valuable products such as proteins, colors, minerals, and flavors can be found in abundance as by-products in fish. Fish mucus, which is a byproduct of fish, is thought to be more valuable and has been found to contain antibacterial proteins. The epidermal mucus in fish is thought to be an essential part of innate immunity and helps keep bacteria, fungi, and parasites from inhabiting the body. Numerous fish species have shown antibacterial activity in their mucus; however, this activity appears to differ from species to species, including rainbow trout, tilapia, and rockfish. It can also be unique to particular bacteria (Hussain et al., 2023b).

There are numerous reasons that amphibians have been studied, including for clinical research, pharmacology, culture, entertainment, and religion (Adil et al., 2022). In order to learn new things about all living things, they have been taken into consideration and utilized as a suitable model (Shivoga et al., 2021). In particular, giant salamanders and frogs have been reared in captivity for human consumption as rare, high-prestige meats (Turvey et al., 2021). Mostly in China, these species have been used as medications in Southeast Asia in addition to being used as a delicacy (Liu, 2021). Widely found in both open and wooded areas, *R. temporaria* is sometimes referred to as the Common Frog in Europe (Jansen van Rensburg et al., 2021). In broad terms, production, processing, distribution, and marketing to different places of consumption result in processing waste or by-products. One common byproduct that can be utilized as a source of collagen is skin. Skin waste from giant salamanders and frogs is known to be an alternative source of collagenous or bioactive peptides with good physicochemical properties and bioactivities, including antioxidative, antimicrobial, anti-inflammatory, and drug delivery (Wang et al., 2025). Numerous BPs, including bradykinin, bombesin, bombinins, caeruleins, secretogranins, dermaseptins, tachykinins, and temporins, have been isolated and examined from the skin of amphibians (Indriani et al., 2023). Research has examined the antibacterial, wound-healing, and antidiabetic qualities of tigerinins derived from *R. tigrina* (Wang et al., 2025). According to a recent study, Asian bullfrog skin can be used to make a collagenous derivative that has great potential for use as a biocompatible material in human food (Cimenoglu, 2021). As species that are sensitive to temperature and humidity, amphibians can react quickly to a variety of environmental changes. The histology of skin matrices can be impacted by this physiological adaptability, which sets it apart from other animal skins (McKnight et al., 2022). For example, salamanders and frogs have a physiological self-defense mechanism that makes their skin release toxins or other bioactive substances when they are in hazardous environments (Ferrell, 2021).

One of the most important medical discoveries ever made has been antibiotics. The contemporary antibiotic era began when Alexander Fleming discovered penicillin in 1929 (Selwyn and Bruivels, 2023). Only with the start of World War II, when the antibiotic was widely employed to treat soldiers' infected wounds, did its

true potential become apparent (Alfawares et al., 2022). Following World War II, antibiotic research and development entered what has been dubbed "The Golden Era" (Muteeb et al., 2023). The number of antibiotic medications approved for clinical use rose steeply during this time. Antibiotic resistance, however, had already started to appear at that early point. When susceptible bacteria are replaced by resistant strains in a municipal setting, antibiotic resistance results (Urban-Chmiel et al., 2022). The rise of organisms resistant to antibiotics and the rise in the prevalence of recently identified harmful bacteria and fungi have partially incomplete the benefits of antibiotic use in public health care (Salam et al., 2023). Antibiotic resistance is recognized as a significant worldwide public health issue since it raises human disease, death, and interrelated health expenditures (Aslam et al., 2021). As a result, the expedition for novel antimicrobial drugs has resumed. As lead molecules for novel antibiotics, antimicrobial peptides have demonstrated potential. Here, we summarize the data on the antimicrobial peptides of new antimicrobial compounds from fish and frog skin secretions. Also, we have provided a detailed their ecological significance and biomedical role in various aspects.

MATERIALS AND METHODS

Molecular secretion strategies, antibiotic resistance, frog and fish antimicrobial activity, antimicrobial peptides, frog and fish secretions, fish secretion, frog and fish skin qualities, and pharmacological significance were among the keywords included in a thorough literature search. Elsevier, ScienceDirect, PubMed, Google Scholar, and Taylor & Francis were used in the search because there were still articles published on this subject from that time period. Following the acquisition of the literature, a thorough analysis was conducted to ascertain the techniques for peptide isolation and extraction, chemical clarification, and biological function testing.

DISCUSSION

PRODUCTION OF FISH MUCUS

Cells such as goblet and sacciform in the fish epithelium produce the mucus matrix (Alsafy et al., 2025). Although it appears that the route of delivery could be retained, except for mucin, none of the other molecules in fish have been extensively studied (Subramanian et al., 2022). Proteins can be supplied to the mucus layer using the traditional extracellular arterial delivery method, in which ribosomes on the rough endoplasmic reticulum (ER) create proteins, which are subsequently transferred to the cell membrane via the Golgi complex (Fröhlich, 2022). Transporters and other small molecules could also carry proteins (which are generated in the cytosol) and other compounds to the mucus layers via transport routes directly over the cell membrane (Zheng et al., 2023). Mucus proteins and other compounds may also originate from dead epidermal cells (Marriott and Gregory, 2024). It is crucial to remember, nevertheless, that chemicals released by cellular debris may still have significant roles in the secretion deposit. A variety of mucus compounds, including secondary metabolites and antimicrobial peptides, may potentially originate from the commensal microbiota community, which includes bacteria and fungi (Fobofou et al., 2022).

FISH MUCUS AND ITS ROLE IN BIOLOGICAL ACTIVITIES

O-glycosylated proteins known as mucins make up the majority of the mucus gel matrix, but they have a wide range of extra substances, including structural, immune-related proteins, antimicrobial peptides, lipids, and smaller molecules like crinotoxins in fish epidermal toxins unrelated to any venom apparatus and MAAs that exhibit a broad range of biological roles (Ndandala et al., 2023). A varied population of bacteria, fungi, and viruses that are found on the mucosal surfaces of fish also contributes significantly to the preservation of host health and homeostasis (Kong et al., 2024). Given that fish mucus serves as the primary defense against infections, all of its constituent molecules and microbiota may work in concert to prevent pathogen entry by utilizing various antimicrobial properties and engaging in immune responses similar to those shown in rats (Sayyaf Dezfuli et al., 2023).

FISH MUCUS AND MOLECULAR APPROACHES

Fish epithelial and mucus layers have long been studied for their molecular distribution using histochemical techniques, including histochemistry, immunohistochemistry, or even electron microscopic cytochemical approaches (Alsafy et al., 2025). Although the use of immunohistochemical techniques offers the benefit of in situ molecule detection without mucus contamination, these methods are only applicable to molecules for which antibodies have been discovered (Mebratie and Dagnaw, 2024). On the other hand, MALDI-IMS eliminates the requirement for target-specific reagents and allows for the investigation of spatial molecular configurations in tissue slices. In contrast to immunochemistry, which usually studies a single antigen, imaging mass spectrometry (IMS) allows for the investigation of conventional histology while simultaneously measuring thousands of analytes (Zhang et al., 2025). This in situ technique's ability to study a wide range of analytes is MALDI-IMS's primary benefit (Shen et al., 2024). The saponins in the sea star mucus have been successfully highlighted by matrix-assisted laser desorption/ionization–imaging mass spectrometry (MALDI-IMS) (Popov et al., 2022). This technique holds the potential to identify new fish mucus molecules, investigate their spatial distribution, and identify new extraction methods.

Research on fish mucus is expanding quickly. The analysis of genes in fish mucus is made easier by advancements in genomics, which also enable the quick growth of microbiomics (microbiome characterization) (Swain et al., 2022). According to a recent study by (Volpe et al., 2025), which sequenced the DNA of European eel skin mucus, fish mucus surfaces serve as natural habitats for the evolution of aquatic mucosal pathogens. An effective method for finding new genes that contribute to mucosal immunity is transcriptomics, a field that involves the study of the entire collection of RNA transcripts produced by the genome, or transcriptome. It can also provide insights into key aspects of mucosal functions, including secretion, microbial pathogenesis, immune responses in the host, and the kinetics of these responses (Zhou et al., 2025). Since well-annotated genomes are still rare in nonmodel and noncommercial fish species, the biological interpretation of RNA-seq (the entire transcriptome sequencing) is still difficult (Li et al., 2025).

After salmon smoltification and after bacterial infections in fish, the mucosal responses have been effectively described using gene expression and transcriptomics

(Krasnov et al., 2021). In addition to revealing that transcription in mucin is delimited through various initiators, as found in Atlantic salmon genetic research, identified seven genes, offering fresh information on mucosal health (Ara-Díaz et al., 2024). To date, the most effective high-throughput method for studying fish mucus has been proteomics (Reyes-López et al., 2021). For commercial fish species, including lump sucker (*Cyclopterus lumpus*), *Dicentrarchus labrax*, *Sparus aurata*, Atlantic cod, and discus fish (*Symphysodon aequifasciata*), several reference skin mucus proteomes have been reported. The pursuit of thorough comparison research to gain a better understanding of fish mucus dynamics is made possible by proteomics. The mucus dynamics of diseased fish, overcrowded fish, chronic stress fish, and fish exposed to various diets have been clarified by proteomics investigations (Natnan et al., 2021).

The study of an organism's tiny molecules, or metabolome, in detail is known as metabolomics, and it offers a snapshot of the organism's status at a given moment in time under particular circumstances (Muthubharathi et al., 2021). For this reason, metabolomics is particularly helpful in understanding the response of organisms and in the search for biomarkers (Araújo et al., 2022). Understanding the metabolome of fish mucus will help not only identify new mucus components but also the roles that fish mucus plays in many processes. The mucus in the skin metabolome of male and female fathead minnows (*Pimephales promelas*) subjected to bisphenol A was shown to be substantially sexually dimorphic (Jeon et al., 2025). The mucus in the gill metabolome of butterflyfishes was examined (Reverter et al., 2022), in connection with many fish characteristics, including geographic location, habitat type, and species taxonomy. They discovered the primary determinant of the mucus metabolome in the gill. Lastly, mucus in fish metabolomics research is still problematic because of the large volume of data collected and the dearth of specialist databases and knowledge on fish metabolites, which makes metabolite identification very complex.

FISH MUCUS AND ITS ANTIMICROBIAL ACTIVITIES

The external mucus of fish contains a variety of antimicrobial molecules, such as pore-forming glycoproteins, enzymes, proteins, and several crinotoxins (Díaz-Puertas et al., 2023). It has also been noted that fish mucus contains antibacterial peptides, one of the primary compounds used to combat infections (Hussain et al., 2023b). The skin mucus of fish has been found to contain several histones with antifungal, antibacterial, and antiparasitic properties (Lee et al., 2023). Several AMPs derived from the terminal portions of histones (Akhavan-Bahabadi et al. 2025), have also been described. Although the processes by which mucus commensal microbiota suppress opportunistic infections are still unclear, they are crucial (Dey and Ray Chaudhuri, 2023). Recent research is demonstrating particular ways by which commensal bacteria could be detected by the host and control pathogen proliferation (Stevens et al., 2021). Until recently, it was believed that commensal bacteria controlled pathogens through mutually competing partnerships. For instance, *Flectobacillus* major from rainbow trout's external mucosal surfaces generated sphingolipids that shaped teleost B cells and antibody responses, stimulated the development of immunoglobulin T (IgT), and controlled the growth of other

symbionts (Yu et al., 2021). Several investigations discovered antifungal and antibacterial properties in bacterial strains isolated from fish mucus, indicating that microorganisms produce specific compounds that may regulate the growth of host diseases (Hussain et al., 2023a).

ROLE OF MUCUS IN CONVENTIONAL MEDICINE

Humans have utilized animals and their parts for therapeutic benefits since prehistoric times (Hardy, 2021). The skin and bodily secretions of animals, as well as animal housing, were frequently used as popular medicines (González and Vallejo, 2021). Traditionally, the Chinese have used toad secretions and frog skin to treat dog bites and control internal body functions and reproduction (Miraaj and Medicine, 2022). Chinese traditional medicine treats Alzheimer's disease by using extracts of the giant leaf frog's scraped skin secretions (Nogueira et al., 2022). During the Vietnam War in the 1960s, the scarcity of adequate medical supplies for treating napalm burns prompted surgeons in Vietnam to explore traditional therapies for scorches. They discovered that using frog skins can be beneficial against severe skin loss and is an effective treatment (Vergneau-Grosset and Weber III, 2021). When applied to Wistar rats, frog skin wounds healed considerably more quickly than cotton gauze wounds. Every two days until full healing took place, biochemical evaluations of wound granulation were carried out. The results indicated that the rats receiving skin grafts formed higher levels of hydroxyproline (Musa and Min, 2022). Hydroxyproline is an essential constituent of collagen, which makes up epithelial tissues (Singh et al., 2023).

AMPHIBIANS AND FROGS

The limbs of anurans include a tongue, a voice box, a sternum, external eardrums, eyelids, skin glands, and fingers and toes (Brito-Zapata et al., 2024). The majority have paired lungs and a three-chambered heart. As cold-blooded animals, frogs and toads rely on their surroundings to warm their ectoderms (Moreno-Rueda and Comas, 2023). The physical characteristics of frogs and toads are not very different. In contrast to toads, which live on land and utilize water for breeding (Lewis, 2022). The parotid glands behind the eyes of toads are big (Maji et al., 2025). Frogs have a thinner waist and body; they have webbed feet for swimming and long hind legs for hopping. Toads, on the other hand, stroll rather than hop, have short hind legs, and have larger, flatter bodies. In terms of anatomy, biochemistry, and physiology, the skin of amphibians is a complex organ that serves a variety of vital purposes for the organism's existence. The frog's skin has a thin, flexible layer that facilitates breathing and water absorption. In addition to facilitating dermal respiration, the skin's abundant blood supply keeps infections out (Teo et al., 2025).

The issue of defense against invasive microorganisms is one that all multicellular creatures face. Bacteria, fungi, and other invaders may be able to enter through the skin (Swaney et al., 2021). Innate immunity, which includes skin glands that may create chemicals poisonous to other animals, is a crucial part of the host-resistance machinery (Silva and Gomes, 2024). These glands can be found concentrated in particular regions of the skin or dispersed across it. The substances that the glands secrete have a variety of uses, including controlling the skin's

physiological processes and protecting the body from infections and/or predators (Praveenkumar et al., 2023). A variety of toxic chemicals produced by the skin glands have the potential to cause morbidity and mortality in mammals. Granules abound in the cytoplasm of epithelial cells, while the lumen is reduced to a tiny space. Through a holocrine process, the glands' contents are released synchronously when the myocytes around them contract (Jacob, 2021). These secretions contain peptides known as antimicrobial peptides (Datta et al., 2021) that can stop the growth of harmful microbes (Soni et al., 2024).

FROG SECRETIONS AND THEIR MEDICINAL PROPERTIES

Amphibians produce strong antimicrobial peptides as a defense because they live in settings that are rich in microorganisms. According to (Johnstone and Herzberg, 2022), the granular glands of the epidermis and nonlymphoid cells on the mucosal surfaces of the gastrointestinal and respiratory systems secrete the antimicrobial peptides. Given that amphibian skin has antibacterial and respiratory properties, some compounds present in secretions could probably be useful in treating respiratory and skin infections (Akat Çömden et al., 2023). The following is an overview of research on frogs, the anuran species that has been examined the most. Peptides that are bactericidal and fungicidal and are produced in epithelial tissues of some frogs are a source of possible medicinal mediators (Pucca et al., 2025). For example, a substance found in a species of frogs belonging to the genus *Rana* was found to be efficient against viruses that are seldom impacted by medications and against *Staphylococcus aureus*, which frequently causes boils and abscesses (Miraaj and Medicine, 2022). Several biologically active substances are present in high concentrations in the African frog skin (Carrillo et al., 2024). It has been demonstrated that these peptides, which are non-hemolytic and water-soluble, inhibit *Candida albicans* (Patil et al., 2023). It appears that the peptides found in *X. laevis* belong to an as-yet-undiscovered family of vertebrate antibacterial peptides.

The antimicrobial peptides of frogs in the *Rana* genus have been the subject of numerous investigations (García et al., 2024). Instead of the Arctic, southern America, and Australia, this genus has more than 250 species that are found all over the world (Morrone and Ebach, 2022). This genus of frogs has been shown to contain a wealth of peptides with antifungal and antibacterial properties. More than 20 *Rana* genus amphibians have been shown to have approximately 160 antimicrobial peptides (García et al., 2024). Peptides were extracted from *Rana pirica*, *Rana japonica*, *Rana tagoi*, and *Rana ornativentris* (Aono et al., 2024). For instance, lytic, linear, cationic, lysine-rich peptides are the dermaseptins that the South American arboreal frog (*Phyllomedusa sauvagii*) produces (Khemaissa et al., 2022). The antifungal substance skin-PYY, which is produced by another South American tree frog (*Phyllomedusa bicolor*), is closely related to gastrointestinal tract peptides and neuropeptide Y. *Aspergillus fumigatus*, *Candida albicans*, and *Cryptococcus neoformans* are inhibited from growing by SPYY, which may penetrate phospholipid membranes (Pruitt et al., 2025). In a study of the pickerel frog's (*Rana palustris*) skin secretions, peptides having various impede effects on bacteria and fungi were isolated (Pucca et al., 2025). These antimicrobial peptides have demonstrated good efficacy against fungus

(*C. albicans*), protozoa (*Leishmania donovani*), and Gram-positive bacteria (Kaushik et al., 2024).

FROG SKIN AS A SOURCE OF BIOACTIVE SUBSTANCES

To obtain meat for human use, amphibian processing typically entails deskinning. High demand will inevitably result in a high by-product, such as skin, as was previously stated. The protein found in amphibian skin, primarily collagen, can be transformed into BPs and collagenous derivatives. A minimum of one bioactivity is present in BPs derived from frog skins and secretions, such as antibacterial, antioxidant, anticancer, and antidiabetic activity (Huyen et al., 2023). Peptides with a variety of bioactivities can be obtained by collecting and purifying the skin secretions of amphibians, particularly frogs, while they are alive. Related BPs' amino acid sequences have been discovered by earlier research on frog skin secretions (Armstrong et al., 2023). In a membrane-mimetic environment, cationic and hydrophobic peptides are assumed to form an amphipathic helix, albeit a clear correlation between the peptide's bioactivity and amino acid composition is still unknown (Cai et al., 2024). Moreover, the health effects of collagen and its derivatives from frog skins are comparable to those of the human body (Feng et al., 2022). Skins from amphibians have been thought of as collagenous supplies for the synthesis of gelatin, collagen hydrolysate (CH), and collagen (Cimenoglu, 2021). Multiple intricate biochemical processes and mechanisms are involved in the biofunctional characteristics of peptides. When they work, these cause peptides to take different actions.

Furthermore, some BPs, like temporin, which has antibacterial and antioxidant properties along with a unique amino acid sequence, may exhibit multiple biofunctionalities (D'Andrea and Romanelli, 2023). Due to their ability to adapt to a variety of habitats, including freshwater and terrestrial situations, amphibians' distinctive BPs are closely correlated with the histology of their skin. Granular and mucus glands in their skin allow them to secrete some powerful BPs and shield them against microbial illnesses and predators (Bhatnagar et al., 2021). However, because BPs' peptide/amino acid sequences change following digestion, these usual bioactivities might change. During stomach digestion, the majority of the proteins are totally broken down, producing new BPs with altered sequences (Lestido-Cardama et al., 2022). The primary tissue used by amphibians for breathing, protection, and excretion is their skin (Akat Çömnden et al., 2023). Additionally, this enables them to produce multifunctional peptides more effectively than other land and marine organisms.

ANTIMICROBIAL PEPTIDES

A system of host-defense mechanisms, which includes a broad-spectrum antimicrobial peptide-based chemical defense system that is non-specific, helps vertebrates' innate immunity against microbial invasion (Sahoo et al., 2021). Antimicrobial peptides are peptides of 10–50 amino acids that are produced by ribosomes and encoded by genes (Hurtado-Rios et al., 2022). Most are made as pre-pro-peptides with a C-terminal peptide (cationic, pro-segment), and an N-terminal signal sequence (Laneri et al., 2021). The majority of anurans release peptides that are

between 1 and 10 kDa in size. Antimicrobial peptides frequently have one or two disulfide bridges and can have a linear, cyclic, or open-ended cyclic structure (Kawmudhi et al., 2025). They exhibit strong amphipathicity toward surfaces that are positively charged as well as hydrophobic (Saha et al., 2022). According to research, these peptides can hinder the evolution of bacteria, fungi, protozoa, and cancer in experimental settings. Although there are still disagreements about the precise mechanism of these peptides, it is understood that their cationic qualities damage cell membranes and lead to uncontrollably high levels of ion exchange with the environment (Gouda et al., 2023). Antimicrobial peptides act at a rate that appears to be far too quick for any process that involves translocation and attachment to an intracellular target molecule, supporting this proposed mechanism (Li et al., 2022). Consequently, the action's speed indicates that the mechanism at play is cell lysis brought on by the peptide's interaction with membrane phospholipids rather than specific cell membrane receptors. On the other hand, the potential therapeutic application of many peptides may be hampered by their toxicity and rapid clearance rate (Lamers, 2022).

Antimicrobial peptides (AMPs) and other bioactive chemicals have been utilized more frequently recently to combat the fast-rising prevalence of bacterial resistance to common antibiotics and chemotherapeutics (Mba et al., 2022). Different methods of action that each AMP experiences based on its structure and specificity. The antibacterial models used by BPs that have an aggregate model that is somewhat resembling the toroidal pore model were proposed by (Li et al., 2024). Nevertheless, there are currently no published linked molecular research on certain antibacterial pathways, especially those involving amphibian BPs. Researchers have merely suggested these pathways as prediction mechanisms. Peptide sequences with positively-charged amino acids can enter bacterial membranes and interact with the phospholipids' negative cell wall charges to cause cellular lysis (Segovia et al., 2021). Moreover, altering the net charge or the hydrophobicity ratio, which is determined by the proportion of hydrophobic amino acids in the peptide sequence, can alter the antibacterial activity of cationic peptides. A higher hydrophobicity ratio corresponds to a higher level of antibacterial activity (Zhang et al., 2022).

It has been shown that the biological activities of small peptides depend on the sequence and content of their amino acids (Apostolopoulos et al., 2021). Toroidal pore, barrel-stave, and carpet models were the only three primary membrane penetration mechanisms found in AMPs belonging to the helix group. But more information on the mechanism is still unknown (Tripathi et al., 2023). According to reports, AMPs derived from the skin and secretions of amphibians have biological actions that inhibit the growth of both Gram-positive and Gram-negative bacteria and fungi. Esculine, brevinine, ranatuerin, ranacyclin, temporin, bombinin, and dybowskin are the main AMPs that have been categorized into various categories based on their presence in amphibian skin secretions (Chen et al., 2022). AMPs generally can suppress the development of *Staphylococcus aureus*, *Candida albicans*, and *Escherichia coli* at a broad range of minimum inhibitory concentrations (MIC). It was not restricted to particular bacterial species, though. AMPs from amphibians, including *Bacterium megaterium*, *Pseudomonas aeruginosa*, and *Bacillus cereus*, inhibited other bacteria. AMPs' molecular diversity had an impact on the MIC. AMPs

have been shown to suppress the bacterial cells' ability to synthesize proteins, including DNA and RNA (Erdem Büyükkiraz and Kesmen, 2022). Table 1 provides numerous antimicrobial peptides and their role.

Table 1: Antimicrobial Peptides.

Sr.	Peptides	Role/Activity	Reference
1	AFP	Anti-Fungal	(Krishnamurthy et al., 2020)
2	Lactocilin	Anti-Bacterial	(Magana et al., 2020)
3	Histatin	Anti-Bacterial/Fungal	(Khurshid et al., 2017)
4	PVD1	Antifungal	(Mello et al., 2011)
5	CecA	Anti-Bacterial	(Wu et al., 2018)

FROG ANTIMICROBIAL PEPTIDES AND MOLECULAR APPROACHES

Although the process of manually sequencing antimicrobial peptides dates back to the 1960s, it is incredibly time-consuming and inefficient, requiring a huge number of specimens, which raises serious ethical concerns in contemporary culture (Mihooliya and Kumari, 2024). Peptides, such as Arg, Val, and Gly, were previously separated using capillary electrophoresis, two-dimensional gel electrophoresis, liquid chromatography, and surface-enhanced laser desorption/ionization (Ahmad et al., 2025). Although circular dichroism spectroscopy and nuclear magnetic resonance can be used for structural elucidation, mass spectrometry has recently gained more attention (Ma, 2022). According to (Snyder et al., 2021), mass spectrometry does not cleave peptides, unlike classical theory, which posits that proteins are broken down into ordered fragments. Instead, it employs complex algorithms to determine the quantity of cleavages and fragments generated, allowing it to speculate as to whether the sequences formed are part of the peptides or the side chains of amino acids. It has been discovered that most skin peptides contain prolyl residues, namely terminal arginyls, and often block N-terminal, altering amidated C-termini. These features would significantly impair the interpretation of MS/MS spectra (Chen et al., 2023). In addition to mass spectrometry investigations, molecular cloning of precursors can be used to confirm the structures of new peptides structurally assigned by Edman degradation (Jayathirtha et al., 2021).

BIOMEDICAL APPLICATIONS

Antimicrobial peptides have been suggested for a variety of medicinal uses (Datta et al., 2021). It is thought that fish and frog skin peptides have cytolytic activity due to the intricate interactions of amphipathic, cationic, α -helical, and hydrophobic properties (Aguilar et al., 2025). They could be used as a chemical to prevent the extent of Neisseria infections and diseases such as AIDS, HIV, chlamydia, and herpes simplex virus (Ikokwu et al., 2023) due to their broad-spectrum effectiveness. The usage of medical equipment like intravenous catheters is significantly vulnerable to

microbial colonization and growth on synthetic polymeric materials. Using magainin peptides, which maintain their antibacterial action when covalently bonded to insoluble polymeric beads, is one possible remedy (Silva et al., 2022). Peptides' antifungal qualities have been researched for about 40 years, and in the last 10 to 15 years, there has been a notable surge in interest in their antifungal potential due to the toxicity of currently prescribed antifungal medications and the rise in fungal infection resistance (Vitiello et al., 2023).

Some MAAs found in fish mucus have a photoprotective effect against sunlight (Singh et al., 2021). As of right now, three distinct MAAs have been found in fish mucus, and different fish species exhibit distinct MAA combinations (Álvarez et al., 2024). Additionally, several investigations have demonstrated the cytotoxic properties of fish mucus against particular cancers, suggesting that fish mucus may be used to generate novel pharmacological antitumoral techniques (Alabssawy et al., 2024). The study of specialized metabolites in fish mucus has been very limited; however, this is expected to change in the next years due to the advent of high-throughput techniques like metabolomics, which may uncover new biological functions of fish mucus.

RESEARCH CHALLENGES IN FISH AND FROG

Examining frogs and fish for various assessments showed numerous difficulties. These difficulties may arise from methodological or ethical issues. The appropriate ethics boards of institutions and conservation groups must grant their approval before any research may begin. The quantity, species, and precise location of the frogs and fish to be gathered must be specified in a permission that must be obtained from the nature protection authorities. This necessity may pose an issue because such information is limited. Additionally, the time of collecting is crucial and could lead to practical issues. Before being taken to the lab, the specimens must be kept overnight in a location that won't infuriate the frogs or allow them to wound themselves. The majority of frogs are gathered at night during the rainy season or close to dams. The techniques employed to obtain the secretions may also have bioethical ramifications after they have been caught. Skin harvesting, chemical stimulation, and electrical stimulation are the three techniques used to collect the secretions.

In earlier research, electrical stimulation was employed. The amphibians do not seem to be harmed by the moderate electrical stimulation used to extract skin fluids. After being carefully cleaned with distilled water from the skin's surface, the secretions are gathered in a glass and lyophilized. Electrical stimulation seems to generate large volumes of secretion; however, the equipment needed for the procedure makes it difficult to use. It has a limited throughput and cannot be used in the field. Additionally, electrical stimulation may cause discomfort (Xu et al., 2021), which raises ethical concerns. Norepinephrine is administered consensually to stimulate the body and cause secretion (Murala and Bollu, 2022). After 21 days, the process is carried out once more. The use of such a banned substance and the requirement for a certain level of specialized technical training are the method's disadvantages. It is also intrusive, and the frogs that are treated can end up dead. A chemical irritant is used as another chemical stimulation technique (Almalty et al., 2024). The procedure has been successfully used and seems to be the least intrusive

and complicated. Some frogs are placed inside a cylinder that has an absorbent cotton piece saturated with anhydrous ether inside. After being exposed to ether for one to two minutes, the frogs' skins release a lot of secretions, which are gathered by washing each animal's dorsal region with a buffer solution.

Although non-destructive and humane stimulation techniques, including electrical and chemical stimulation, are used, skin harvesting entails killing the frogs and then removing their skins (Kraft, 2023). Solid phase extraction and homogenization are used to obtain the secretions. Conservation authorities are unlikely to authorize such experiments due to the significant ethical issues raised by this technology, particularly in metropolitan regions where frog populations are already in danger. Peptide yields may also be decreased as a result of the extraction procedure. In order to prevent endopeptidase action, the secretions should always be put on ice as soon as they are collected. The supernatant can then be centrifuged and lyophilized to continue the compound extraction procedure. Because of the often modest returns, it is highly advised to utilize a large number of animals. Once the animals have received care for at least 24 hours, they can be returned to their natural habitat. Last but not least, despite being a significant difficulty at the moment, integrating several omics methodologies will boost studies on mucus in fish. Discovering pathways and processes that would otherwise go unnoticed is made possible by omics integration.

LIMITATIONS OF SECRETION USE IN CLINICAL DRUG

The conversion of peptides into therapeutic contenders has so far seen a dull outcome, despite the optimistic image as presented in the previous discussion (Zheng et al., 2025). Due to issues with toxicity and safety or poor clinical results, none of the seven peptide medications that were in prosecutions during the previous ten years have received FDA approval. Antimicrobial peptides' efficacy and mechanism of action make them tempting therapeutic agents (Odunitan et al., 2025). Their disruption of membranes, however, can result in both local and non-selective systemic toxicity. Magainin derivatives, for instance, have been demonstrated to prevent pregnancy establishment in monkeys when administered intravaginally due to their binding to placental trophoblast cells (Virgilio et al., 2024).

The stability of formed peptides, their unclear biological actions, and the possibly high manufacturing costs are among the non-pharmacological reasons for failure that have been mentioned (Tonin and Klen, 2023). Antimicrobial peptide research and development are still in their early stages and have great potential for the future, despite the present worries and difficulties. Although amphibians offer a possibility to create BPs with high functions and health benefits, some key elements should be addressed before their use in the food and biomedical industries. The following limitations are mentioned in this review such as food safety and health, culture and religion, animal welfare and the environment, and sustainable production (Muhammad et al., 2022). Therefore, a transdisciplinary, cross-sectoral approach is needed to assess the utilization of amphibian meat and skin. Because of enzymatic processes, lipid oxidations, and microbial activity, amphibian skin possesses distinct and unpleasant odorants that affect the quality of food (Chen et al., 2021). For example, CGS's skin smells stuffy, dirty, medicinal, rancid, and unsophisticated.

Depending on the extraction technique, the collagen isolated from CGS skin has a different odorant profile. While the pepsin-soluble collagen exhibited a low intensity of off-odors, the acid-soluble collagen detected sour, ammonia-like, and bitter off-odors (Chen et al., 2021).

CONCLUSIONS AND FUTURE PERSPECTIVES

It has been investigated to use amphibian skin and secretions as a substitute source of collagen and bioactive peptides. They offer a variety of exceptional bioactivities for innovative BPs. Bioactivities such as antibacterial, antioxidant, antidiabetic, and antiviral are all present in the isolated peptides. Several amphibian species contributed to the length and uniqueness of the amino acid sequence. There are a few advantages and disadvantages of eating amphibians and items derived from them. While there is a lot of potential for using this BP source, extraction should be limited to prevent amphibian extinction and indirectly preserve the ecosystem. Thus, with appropriate handling, amphibians, especially frogs and giant salamanders, can be used as a practical source to enhance human health and well-being. The significance of secondary metabolites, their diverse bioactivities in more thoroughly researched species. Moreover, the advancement of novel fields will make it possible to research secondary fish metabolites more effectively. In order to better understand the mucus and its biological and ecological roles, as well as to find new metabolites, research into the microbiome and mycobiome of fish mucus is a potential area. Microbial infections are becoming more resistant to antibiotics due to abuse and the consequent natural selection of resistant variants. As lead molecules for antimicrobial research and development, novel pharmacophores must be introduced. Substances that have a defensive role are abundant in amphibian and fish skin. Apart from various peptides that have counterparts in mammals, amphibians, and fish, a secretion also has peptides against microbes.

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Competing interests: Authors have declared that no competing interests exist.

Funding: Authors have no source of funding for this work.

Authors' contributions: Sohail, Kamran, Mansha and Nawaz have designed this project, collected data and written this article; while Fozia has critically analyzed this article and approved as final.

