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Review Article

A Systematic Review of Antimicrobial Peptides from Fish with Anticancer Properties

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ABSTRACT

Fish is a potential source of various forms of bioactive compounds. It can be used as a source of specific proteins, especially in medicine. Recently, studies related to the use of antimicrobial peptides (AMPs) from fish are being carried out to find an alternative cure for cancer. To achieve this objective, the AMP used must meet a condition where it possesses the ability to target tumor cells without affecting the normal cell. Therefore, this study aims to systematically review and classify the recent AMPs isolated from fish and their therapeutic activities, focusing on their anticancer and antimicrobial activities. A systematic review of studies published in English between 2017 and 2020 was conducted in PubMed NCBI, Biomed Central, Science Direct, and Google Scholar databases using keywords and inclusion and exclusion criteria. A systematic review conducted has identified 38 potential AMPs isolated from fish that have been reported to have antimicrobials activity. Of all these, 21 AMPs also have anticancer properties.

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Therefore, it is important to continue to explore and study natural ingredients in developing new approaches in medicine. This research is essential to enable the potential of AMPs to be identified and applied.

Keywords: Antimicrobial peptides, antitumor activity, fish, medicinal agents

INTRODUCTION

Antimicrobial peptides (AMPs) are low molecular weight proteins with extensive antimicrobial and immunomodulatory activities against infectious bacteria, viruses, fungi, and cancer. AMPs are diverse proteins classified into subgroups based on their structure and composition of amino acids and other physicochemical properties such as net charge, secondary structural contents, and solubility (Boparai & Sharma, 2019).

Recent studies indicate that AMP has another function besides inhibiting microbes, where they act to repress cancer cells (Boparai & Sharma, 2019). The AMPs have been speculated to target cell membrane molecules, disrupting intracellular signaling. It directly enhances their effectiveness compared to single-target drugs (Neshani et al., 2019). The failure of certain cells to resist AMPs has made it an important drug that will significantly change modern antimicrobial use. In addition to certain specific characteristics, such as short lengths of 10–50 amino acids, cationic existence with a net charge of + 2 to + 11, amphipathicity, and a large proportion of hydrophobic residues (Boparai & Sharma, 2019). AMPs were categorized as cationic or non-cationic depending on their amino acid composition; α-helical, β-sheet, expanded or looped regarding their secondary structure (León et al., 2020). Most of them fall into cationic and either α-helical or β-sheet.

AMPs are present in a wide variety of species within the innate immune system. Since fish live in microbial-rich aquatic ecosystems, they are highly dependent on AMP as their natural immune system. Several peptides have been extracted from fish over the past thirty years and show specific biological activities. Felício et al. (2017) have reported that protein hydrolysis of unicorn leatherjacket skin using collagenase at 5° C successfully extracts collagen peptides that have anticancer, antidiabetic, and wound healing properties. This extract is composed of various molecules belonging to the defensin, cathelicidin, and hepcidin families, as well as a unique family, included exclusively in fish, called piscidin (homologous to cecropin). Fish AMPs are active against fish-specific bacterial and viral pathogens (Buonocore et al., 2019).

Various AMPs extracted from fish have shown a dual function as an antimicrobial and anticancer agent. Several studies showed that the skin mucus of fish has a cytotoxic effect in human cancer cells due to their ability to inactivate a wide range of cancer cells (Deslouches & Di, 2017). One of the most reviewed AMPs is Pardaxin A, extracted from various fish species such as *Red sea bream*, *Salmo salar,* and *Pardachirus marmoratus*.

Pardaxin A inhibits various cancer cell lines, including HT1080T, Hela, HP-1, and U937, through apoptosis, inducing maturation and differentiation of cells (Cheng et al., 2020; Kang et al., 2018).

Fish-isolated peptides are a significant source of pharmacological compounds that can provide resources for academic and practical study (Cipolari et al., 2020). The present work aimed to systematically review the fish AMPs and their therapeutic activities, focusing on their anticancer and antimicrobial activity. The detected peptides were classified according to their pre-established groups and reviewed their sources and activity.

MATERIALS AND METHOD

This systematic review was applied in compliance with the Preferred Reporting Items for Systematic Reviews and Meta-Analysis (PRISMA) (Moher et al., 2009), aimed to address the following research question: What are the activities and classification of AMPs isolated from fish?

Search Strategy

Four electronic databases were searched in February 2020 and updated in January 2021. These included PubMed NCBI, Biomed Central, ScienceDirect, and Google Scholar. Google Scholar was searched to specify any potentially relevant grey literature. The advance search was performed with a combination of words or phrases using Boolean operators (‘AND,’ ‘OR’). The following keywords or MeSH (Medical Subject Headings) were used: “peptide” OR “antimicrobial peptide” OR “bioactive peptide” AND “antitumor” OR “anticancer” OR “antiproliferative” AND “fish” OR “fishes.” Truncations, parentheses, and quotation marks were used whenever possible for advanced search.

Eligibility Criteria

Authors have reviewed the studies against eligibility criteria: (1) The study’s type, year, and language: A research paper (e.g., in vitro, in vivo) and a review paper (e.g., systematic review, scope review, and literature review) published in an indexed journal in English between 2017 and 2020. Studies published in a language other than English before 2017 were excluded. Conference proceedings, books, and dissertations were also excluded. (2) The focus of the studies: The article topic should involve the antimicrobial peptide from fish mucus that shows antitumor or antimicrobial properties. Articles containing synthesized peptides or peptides with no anticancer or antimicrobial properties and derived from sources other than fish mucus were excluded. (3) Quality of the studies: articles should involve appropriate design and high quality as described in the quality appraisal section. The excluded articles either failed the eligibility appraisal or were irrelevant.

Search Outcomes

A total of 3,276 studies were identified from the four electronic databases, as shown in the PRISMA flow diagram (Figure 1). After excluding the duplicated studies (n= 385) and not scientific manuscript between the study period, 551 articles were left (Figure 1). From this number, 379 articles were eliminated after the title and abstract screening. A full text of 172 journal article was screened, of which 135 was excluded as they involved synthesized peptides (n= 69), not associated with anticancer or antimicrobial properties (n= 16), sources other than fish mucus (n= 46), and failed in quality appraisal (n= 4).

Data Extraction and Quality Appraisal

The multiple-assessment approach was conducted between the two authors for studies identification, screening, and selection based on PRISMA guidelines (Moher et al., 2009). The screening, analysis, and synthesized processes were iterative and regular discussions between the research team. Then, the articles selected were assessed using the Guide Evaluation of Qualitative Research Studies by Russel and Gregory (2003). Methods Guide for Medical Test Reviews of the Agency for Healthcare Research and Quality (AHRQ), the Scientific Resource Centre, and the Evidence-based Practice Centers (EPCs), were used to evaluate the observational and clinical studies (Munn et al., 2015; Chang et al., 2012). The assessment tools provided a consistent approach for assessing the reviewed studies (Chang et al., 2012; Russel & Gregory, 2003). Articles with less than 70% of the assessment criteria were categorized as embodying high bias or risk (Walsh & Downe, 2006). Two authors also conducted the quality-appraisal approach of the current study to check for the consistency and reliability of studies (Moher et al., 2009; Walsh & Downe, 2006). A summary of the included articles was presented in Table 1, which shows the study objective, method design, biological sample, results, and reference and year of the study.

RESULTS AND DISCUSSION

After proper selection and screening, 37 articles (Figure 1), consisting of 18 reviews paper, one systematic review, and 18 experimental studies, were reviewed. From this number, 26 articles are about multifunctional peptide activity for individual studies, six about AMPs activity, and five about the anticancer activity. These studies were classified further, where 21 studies focused on fish species, and 16 studies were conducted on various types of marine animals, including fish (Table 1).

Fish Antimicrobial Peptides

The reviewed evidence highlighted the definitions, types, mechanisms of action, benefits, and sources of the AMPs. AMPs are known to be one of the primary defense components

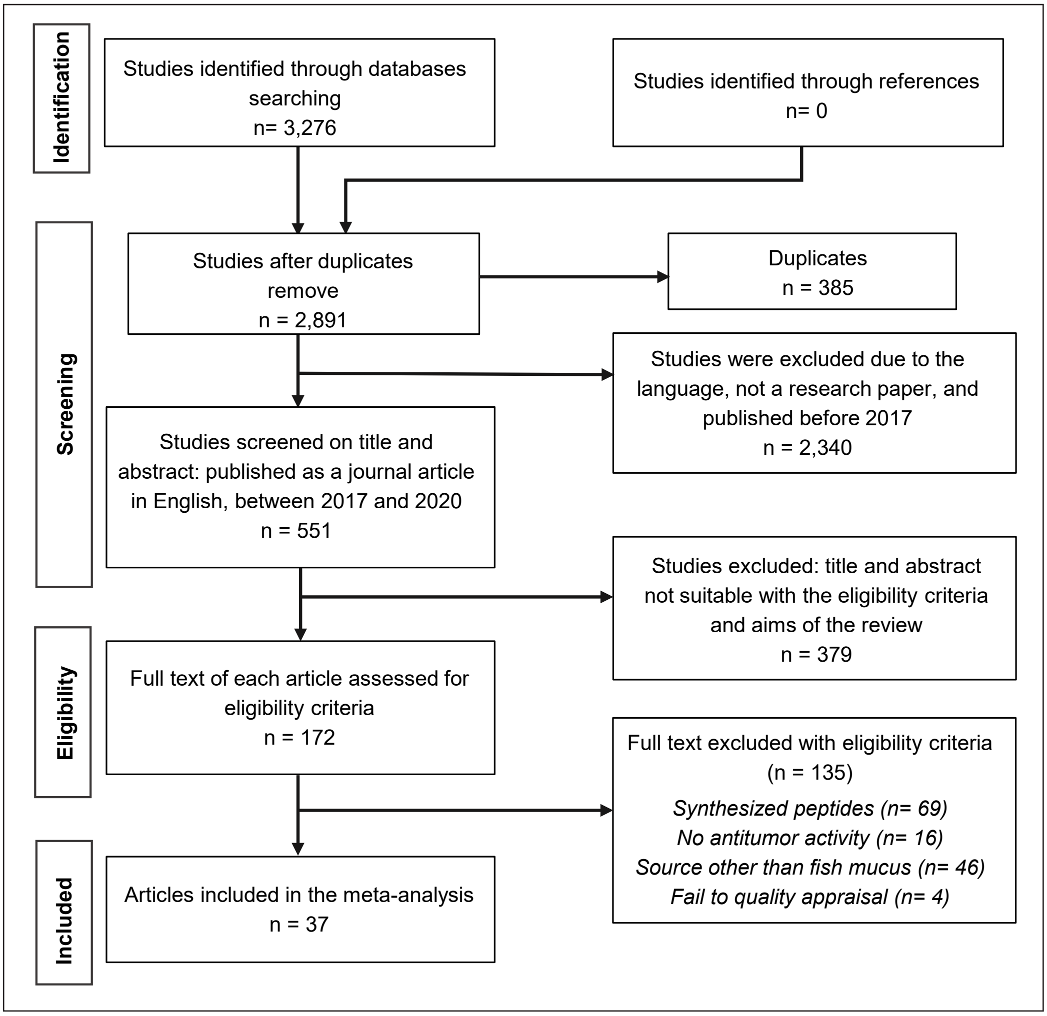

Figure 1. PRISMA flow diagram of antimicrobial peptides with antitumor property isolated from fish mucus

of all animals, such as marine animals, that provide the first protection against various types of microbial pathogens (Devi et al., 2019; Shabir et al., 2018; Valero et al., 2020). In both plant and animal worlds, hundreds of AMPs have been found to date (Shabir et al., 2018; Valero et al., 2020). Among those, fish is an essential source of biologically active peptides and proteins (Devi et al., 2019; Kumar et al., 2019; Lirio et al., 2019). As fish live in a microbe-laden environment and are exposed to be potential pathogenic microorganisms, mucus rich in AMPs was excreted to protect against worm, bacterial and fungal infections (Valero et al., 2020).

This systematic review disclosed 22 fish species reported in the in-vitro studies as a source of AMPs, including *Oreochromis niloticus*, *Clarias batrachus*, *Channa striata* (Chee et al., 2019; Lugo et al., 2019; Raju et al., 2020), *Cyprinus carpio*, *Neogobius melanostomus*

Table 1
Summary of the reported antimicrobial peptide from the reviewed studies

References	Study Objective	Methods	Biological Sample	Main findings	Activity
(Ardershir et al., 2020)	To evaluate the bioactive properties of crude skin mucus derived from two fish species, including common carp (*Cyprinus carpio*) and round goby (*Neogobius melanostomus*).	Experimental method using MTT[1] assay	Species: *Cyprinus carpio* and *Neogobius melanostomus* Extracts: Fish skin mucus	The skin mucus derived from fish mucus has cytotoxic effects on cancer cell lines, including human prostate adenocarcinoma cells (LNCaP) and breast cancer cells (MCF7).	Anticancer
(Hansen et al., 2020)	To isolate the novel cysteine-rich antibacterial peptides, turgencin A and turgencin B, from the Arctic marine colonial *ascidian Synoicum turgens*.	Experimental method using MTT[1] assay and MIC[2]	Species: *ascidian Synoicum* Extracts: Two peptides	The cysteine-rich antibacterial peptides showed activity against both Gram-negative and Gram-positive bacteria and inhibited the growth of the melanoma cancer cell line (A2058) and human fibroblast cell line (MRC-5).	Antimicrobial and anticancer
(Hazam & Chen, 2020; Pan et al., 2017)	To investigate the anticancer activity of five known peptides of *Oreochromis niloticus,* including Tilapia piscidin 1 (TP-1), Tilapia piscidin 2 (TP-2), Tilapia piscidin 3 (TP-3), Tilapia piscidin 4 (TP-4), and Tilapia piscidin 5 (TP-5).	Experimental method MIC[2] and MTT[1] assay	Species: *Oreochromis niloticus* Extracts: Five peptides	Among these peptides, TP4 has shown anticancer against glioblastoma cell lines (U87MG, U251), antimicrobial, and immunomodulatory properties. The TP4 of *Oreochromis niloticus* is a promising candidate for therapeutical application.	Antimicrobial and anticancer
(Kuo et al., 2018)	To study the apoptosis mechanism resulting from MSP-4 (AMP[3]) of *Oreochromis niloticus* in osteosarcoma MG63 cells.	Experimental MTT[1] assay and Western blot	Species: *Oreochromis niloticus* Extracts: MSP-4 peptide	The peptide MSP-4 significantly induced apoptosis in MG63 cells through intrinsic and extrinsic pathways. MSP-4 provides a potentially innovative to the treatment of human osteosarcoma.	Anticancer
(Sruthy et al., 2019)	To characterize the molecular and functional mechanisms of histone H2A derived AMP[3] from the *Fenneropenaeus indicus*.	Experimental MTT[1] assay and MIC[2]	Species: *Fenneropenaeus indicus* Extracts: Histone H2A peptide	The H2A peptide exhibited antimicrobial activity against Gram-negative and Gram-positive bacteria and anticancer activity against lung cancer cells (NCI–H460) and laryngeal carcinoma (HEp-2).	Antimicrobial and anticancer

Table 1 *(continue)*

References	Study Objective	Methods	Biological Sample	Main findings	Activity
(Uen et al., 2019)	To evaluate the anticancer activity of Pardaxin, an AMP[3] isolated from the *Pardachirus marmoratus*, against leukemic THP-1 and U937 cells.	Experimental MTT[1] assay and Western blot	Species: *Pardachirus marmoratus* Extracts: Pardaxin peptide	Pardaxin induced maturation and cell differentiation of leukemia cells into macrophage-like cells with phagocytotic ability. Thus, pardaxin has therapeutic potential for human leukemia.	Anticancer
(Neshani et al., 2019)	To Evaluate the prospect of the application of AMP[3] to cancer therapy, with an emphasis on cationic AMP[3] modes of action.	Review study	Species: Various sources, including fish	The multifunctionality determined by a conventional AMP[3] structure indicates that no single property in natural AMPs[3] is fully optimized to retain multiple functions.	Anticancer
(Felício et al., 2017)	To study characteristics of small peptides that have dual activity against microbial and cancer.	Review study	Species: Various species of fish	As potential chemotherapeutic agents, AMPs[3] that can also serve as an anticancer are considered.	Antimicrobial and anticancer
(León et al., 2020)	To examine and compare the immunological and biochemical properties of skin mucus of three brackish water fishes.	Well diffusion method	Species: *Lates calcarifer, Chanos chanos and Mugil cephalus* Extracts: Fish mucus	*C. chanos* mucus, compared to the other two fishes, has more potent innate immune properties. Thus, the polyculture of this fish with other fish or shrimp species may have beneficial effects for disease prevention.	Antimicrobial and hemolytic activity
(Felício et al., 2017)	To compare peptide limitations and to increase their therapeutic effect.	Review study	Species: Various sources, including fish	A promising and modern approach to the treatment of many diseases, including cancer, is therapeutic peptides.	Antimicrobial and anticancer
(Cipolari et al., 2020)	To discuss the potential of fish by-products as sources of bioactive peptides with anticarcinogenic.	Review study	Species: Fish by-products Extract: various parts "by-products."	To date, a limited number of cell-based and *in vivo* studies have been performed on the antiproliferative and antioxidant activity of peptides from fish by-products.	Anticancer and antioxidant

Table 1 *(continue)*

References	Study Objective	Methods	Biological Sample	Main findings	Activity
(Buonocore et al., 2019)	To find a fast, efficient and straightforward way to obtain the target peptides.	Review study	Species: Marine animal, including fish	Techniques for using organic synthesis for marine bioactive peptides, microwave purification of marine bioactive peptides	Antimicrobial and anticancer
(Deslouches & Di, 2017)	To review the activity of AMPs[3] of fishes as a board antimicrobial spectrum.	Review study	Species: Various species of fish	AMPs[3] can serve as multifunctional peptides to provide a system of a dominant defense.	Antimicrobial
(Kumar et al., 2019)	To have valuable information on the marine potential anticancer activity for human therapy.	Review study	Species: Marine animal, including fish	Marine peptides are chemically diverse, have a wide variety of therapeutic operations, and are highly cell or tissue-specific.	Anticancer
(Neshani et al., 2019)	To hydrolyze the skin of the *unicorn leather jacket* at three different temperatures using collagenase.	The tangential flow filtration system	Species: *Aluterus Monoceros* Extracts: Collagen peptide of skin mucus	At low hydrolysis temperatures (50°C), collagen peptides extracted from fish skin displayed more bio-active behaviors than those hydrolyzed at high temperatures (500°C).	Anticancer, antidiabetic, anti-inflammation and wound healing
(Marqus et al., 2017)	To examine the antimicrobial function of mucus from species of freshwater fish.	MIC[2] and Disc diffusion method	Species: *Oreochromis niloticus, Clarias batrachus, and Channa striata* Extracts: Fish mucus	The current findings revealed the possible antimicrobial use of freshwater fish mucus against pathogens of medical significance.	Antimicrobial
(Nurdiani et al., 2016)	To compare therapeutic drugs' activity in a cell line lung cancer and the peptide's antiproliferative effect.	MTT[1] assay and PSI-BLAST	Species: *Clarias gariepinus* Extracts: Peptide of fish skin	Not only does Catfish PACAP (Pituitary cyclase-activating polypeptide) have inhibitory effects on pathogen development, but it also affects the proliferation of the H460 line of human non-small lung cancer cells.	Antimicrobial and anticancer

Table 1 *(continue)*

References	Study Objective	Methods	Biological Sample	Main findings	Activity
(Wang et al., 2017)	To describe the systems, functions, and putative processes of the prominent fish AMP[3] families.	Review study	Species: Various sources, including fish	AMPs[3] demonstrate that a wide variety of pathogens, such as bacterial, fungal, and viral, are quickly neutralized. AMPs[3] also exhibit numerous biological effects in animals, such as endotoxin neutralization, immunomodulation, and angiogenesis induction.	Antimicrobial, anticancer, antiviral, and antifungal
(Chaturvedi et al., 2020)	To discuss the new methodologies and latest research on fish AMP[3] functions and their future applications in aquaculture and human health care.	Review study	Species: Various species of fish	Antimicrobial drugs can be used directly to defend against pathogens as an active drug complex or indirectly to modulate their immune response and as adjuvants to improve the function of vaccines.	Antimicrobial, antiviral, antifungal, and antiparasitic
(Kang et al., 2018)	To summarize the recent developments in the production of AMPs[3] in food, medicine, and animal applications.	Review study	Species: Various sources, including fish	AMPs[3] exert antimicrobial activity mainly by membrane disruption mechanisms, so they are less likely to cause drug resistance.	Antimicrobial, anticancer antiviral, antifungal, and antiparasitic
(Lirio et al., 2019)	To illustrate the different methods of preparation, biological activity, and associated mode of action of bioactive peptides from fish skin.	Review study	Species: Various species of fish Extracts: Fish skin	Small peptides possess biological activities based on their amino acid composition and sequence.	Antioxidant, anticancer, antihypertensive, antimicrobial, and anti-Alzheimer's
(Lugo et al., 2019)	To describe and characterize Four NK-lysin-like transcripts of Atlantic salmon.	Review study	Species: *Salmo salar* Extracts: cathelicidin 1 peptide	Peptides derived from NK-lysine and cathelicidin 1 are capable of Modulated immune reactions.	Antimicrobial activity and immunomodulatory properties
(Shabir et al., 2018)	To report the first mud crab series of arasin, designated as Ss-arasin.	Complete cDNA sequences	Species: *Scylla serrata* Extracts: Arasin peptide	Antimicrobial action against three species of bacteria and inhibition of the development of human cervical and colon carcinoma cells.	Antimicrobial and anticancer

Table 1 *(continue)*

References	Study Objective	Methods	Biological Sample	Main findings	Activity
(Valero et al., 2020)	To review different physiological effects from peptides and discuss the impact of processing on the peptides.	Review study	Species: Marine animal, including fish Extracts: Fish peptides	The peptides extracted from fish play a significant role in the production of different functional foods and the treatment of chronic diseases.	Antimicrobial and anticancer
(Wang et al., 2019)	To identify the significant AMPs[3] in rockfish, evaluate their antimicrobial behavior, and assess their possible therapeutic use.	Real-time quantitative PCR (RT-qPCR)	Species: *Sebastiscus marmoratus* Extracts: Novel AMPs[3]	AMPs[3] derived from *S. marmoratus* tend to be possible therapeutic applications for aquaculture pathogen infections.	Antimicrobial
(Buonocore et al., 2019)	To design a mutant starting from the Cnd series (Cnd-m3) and to enhance its antimicrobial activity against human pathogens.	TEM[4] immunogold method	Species: *Chionodraco hamatus* Extracts: Cnd-m3 peptide	Relatively low hemolytic activity and poor cytotoxic effects of the Cnd-m3a peptide against human primary and tumor cell lines were observed, but strong antimicrobial activity against selected Gram-human pathogens.	Antimicrobial, anticancer, and hemolytic activity
(Aubine et al., 2019)	To understand the pharmacological properties and future uses of epinecidin-1.	Review study	Species: *Epinephelus coioides* Extracts: epinecidin-1 peptide	The epinecidin-1 peptide of *Epinephelus coioides* proved pharmacological activities.	Antimicrobial, immunomodulatory, Anticancer and wound healing
(Acosta et al., 2019)	To assess the antibacterial activity extract of freshwater fish species against human pathogens, including *Aeromonas hyprophila* and *Staphylococcus aureus*.	Disc diffusion method	Species: *Heteropneustes fossilis*, *Cirrhinus mrigala*, and *Azadirachta indica* Extracts: leaves and bark	The study highlights the importance of fish integumentary and neem extracts as potential antibacterial agents.	Antimicrobial
(Anju et al., 2019)	To study the biotechnological of AMGAP peptide due to the presence of the antibacterial.	Mass spectrometry	Species: *Scomber scombrus* Extract: AMGAP peptide	Antibacterial activity of AMGAP peptide was detected against both Gram-negative and Gram-positive bacteria.	Antimicrobial

Table 1 *(continue)*

References	Study Objective	Methods	Biological Sample	Main findings	Activity
(Bhandari et al., 2020)	To study the antimicrobial and anticancer of the marine peptide.	Review study	Species: Marine animal, including fish	Marine peptides have biological activities, including antibacterial, antifungal, antiviral, cytotoxic, neurotoxic, anticoagulant, antidiabetic, antifreeze, endotoxin-binding, and immune-modulating.	Antifungal, antiviral, antimicrobial, and anticancer
(Boparai & Sharma, 2019)	To discuss applications of AMPs[3] in the treatment of infectious diseases.	Review study	Species: Various species of fish	Peptides extracted from fish display improved effectiveness, high specificity, reduced drug interaction, low toxicity, biodiversity, and direct attack properties.	Antimicrobial and anticancer
(Bo et al., 2019)	To identify the effects of piscidin-1 on species of mitochondrial reactive oxygen and apoptosis in OSA cells.	MTT[1] assay and Disc diffusion assay	Species: *Morone saxatilis* × *M. chrysops* Extracts: Piscidin-1 peptide	The piscidin-1 peptide can inhibit bacterial infections and has the properties of antinociception and anticancer against the OSA bone cell line.	Antimicrobial and anticancer
(Cipolari et al., 2020)	To analyze the data on fish bioactive peptides from 1986 to 2019.	Systematic review	Species: Various species of fish Extracts: Fish skin and sting	Antihypertensive, immunomodulatory and antioxidant, antitumor, and antimicrobial activities have been found in studies on the pharmacological effects of fish peptides.	antihypertensive, immunomodulatory, antioxidant, antitumor, and antimicrobial
(Chee et al., 2019)	To discuss the benefits of AMPs[3] and identify their mechanism of action and improvement in AMP[3] production for use in the treatment of NSCLC.	Review study	Species: Various species of fish	As anticancer drugs, AMPs[3] have gained great interest as they can selectively destroy cancer cells, but not healthy cells. Besides, AMPs[3] show minimal toxicity and minimal chances of resistance development.	Antimicrobial and anticancer

Table 1 *(continue)*

References	Study Objective	Methods	Biological Sample	Main findings	Activity
(León et al., 2020)	To review the *in vitro* testing of the future use of several peptides as antimicrobial agents for humans and fish.	Review study	Species: Various sources, including fish	Peptides have high *in vitro* antibacterial and antiviral activity against major fish pathogens and point to their use in aquaculture as potential therapeutic agents.	Antiviral and antimicrobial
(Devi et al., 2019)	To evaluate the characterization and antimicrobial function of bioinformatics of two piscidin (Pi) peptides known from fish.	MIC[2] and Antibiofilm assays	Species: *Channa striatus* Extracts: Two piscidin (Pi) peptides	The expression of the gene plays a remarkable role in the up and down-regulation during infection; CsPi is involved in innate immunity. The participation of peptides in the formation of antibiofilm and destruction of the bacterial membrane supports its immune character.	Antimicrobial

[1]MTT assay is a method to determine the viability of cells; [2]MIC: a minimum inhibitory concentration; [3]AMP/AMPs: antimicrobial peptide/s; [4]TEM: Transmission electron microscopy

(Abuine et al., 2019), *Ascidian Synoicum* (Hansen et al., 2020), *Fenneropenaeus indicus*, *Salmo salar* (Sruthy et al., 2019), *Pardachirus marmoratus* (Wang et al., 2017), *Lates calcarifer*, *Chanos chanos*, *Mugil cephalus* (Kumar et al., 2019), *Aluterus Monoceros* (Neshani et al., 2019), *Clarias gariepinus* (Lugo et al., 2019), *Scylla serrata* (Anju et al., 2019), *Sebastiscus marmoratus* (Bo et al., 2019), *Chionodraco hamatus* (Buonocore et al., 2019), *Heteropneustes fossilis*, *Cirrhinus mrigala*, *Azadirachta indica* (Devi et al., 2019), *Scomber scombrus* (Offret et al., 2019), and *Morone saxatilis* × *M. chrysops* (Cheng et al., 2020). More fish species (n= 27) were identified in the review studies as a potential source of AMPs, including Codfish, Plaice (*Pleuronectidae*), Groupers, *Pleuronectes americanus* (Shabir et al., 2018; Valero et al., 2020; Chaturvedi et al., 2020), *Oncorhynchus mykiss*, *Danio rerio* (Cipolari et al., 2020; Chaturvedi et al., 2020), *Morone chrysops*, *Channa maculate* (Valero et al., 2020), Red sea bream (Wang et al., 2017), *Grammistes sexlineatus*, *Pogonoperca punctata*, *Ictalurus punctatus*, *Mimetes saxatilis*, *Oncorhynchus mykiss* (Cipolari et al., 2020; Valero et al., 2020), *Epinephelus coiodes*, *Hippoglossus hippoglosus*, *Misgurnus*, *Onchorhynchus mykiss* (Shabir et al., 2018), *Blue whiting* (Wang et al., 2017), *Myxine glutinosa*, *Potamotrygon*, *Ictalurus punctatus*, *Pelteobagrus fulvidraco*, *Thalassophyte nattereri*, *Hybrid fish* (Deslouches & Di, 2017), *Setipinna taty* and *Epinephelus coioides* (Shabir et al., 2018).

On the other hand, most of the reviewed studies (n= 19) reported that the AMPs were isolated from various organs of fish like muscles, bones, and fish by-products (Deslouches & Di, 2017; Kunda, 2020; Marqus et al., 2017; Nurdiani et al., 2016; Wang et al., 2018). These AMPs showed various biological benefits such as antimicrobial, anticancer (Sruthy et al., 2019; Deslouches & Di, 2017; Felício et al., 2017; Marqus et al., 2017; Wang et al., 2017), antifungal, antiviral (Chee et al., 2019; Kumar et al., 2019), antiparasitic (Valero et al., 2020; Wang et al., 2018), antioxidant (Nurdiani al., 2016), immunomodulatory (Chee et al., 2019), wound healing (Chee et al., 2019), and hemolytic (Buonocore et al., 2019). The AMPs isolated from fish skin was reported in 11 studies that showed antimicrobial, anticancer (Deslouches & Di, 2017; Marqus et al., 2017; Kang et al., 2018; Felicio et al., 2017; Wang et al., 2017; Varier et al., 2018), immunomodulatory (Acosta et al., 2019; Cipolari et al., 2020), anti-inflammation (Neshani et al., 2019) and wound healing (Neshani et al., 2019), antihypertensive (Abuine et al., 2019), anti-Alzheimer's (Abuine et al., 2019), and antioxidant (Chee et al., 2019) activities. The fish mucus was also an essential source for AMPs that showed antimicrobial (Buonocore et al., 2019; Chee et al., 2019), anticancer Buonocore et al., 2019; Chee et al., 2019), and hemolytic activity (Kumar et al., 2019) properties (Table 1).

Fish AMPs with Antibacterial and Antiproliferative Activity

This systematic review yielded 21 AMPs with anticancer activity (Table 2). Pardaxin A was the most reviewed peptide (six studies) exhibited anticancer properties. Pardaxin A

Table 2
Summary of the reported antimicrobial peptides extracted from fish and their antitumor mechanisms

AMP	Sources	Anticancer activity	Cell line	Reference
TFD100	Blue whiting and Codfish	Apoptosis, antiproliferative, inhibited adhesion, and angiogenesis	HUVECs	(Wang et al., 2017)
Turgencin A	*Ascidian synoicum*	Antiproliferative	A2058 and MRC-5	(Wang et al., 2017; Shabir et al.,2018), (Kunda, 2020; Chee et al., 2019)
Turgencin B	*Ascidian synoicum*	Antiproliferative	A2058 and MRC-5	(Wang et al., 2017; Deslouches & Di, 2017)
Epinecidin-1	Codfish, Grouper, Plaice, and *Epinephelus coioides*	Antiproliferative and cell membrane lysis by necrosis	HTC and STC	(Wang et al., 2017), (Shabir et al., 2018), (Deslouches & Di, 2017; Kang et al., 2018)
Chrysophsin-1 A	*Red sea bream*	Modulates the inflammatory response and membrane perturbation	HeLa and A549	(Wang et al., 2017)
Pardaxin A	*Red sea bream, Salmo salar, and Pardachirus marmoratus*	Antiproliferative, membrane perturbation, apoptosis, induce maturation, and differentiation	Hela, HT1080, THP-1 and U937	(Shabir et al., 2018)
TH1-5 TH2-2 TH2-5 TH2-3	*Oreochromis niloticus*	Cytotoxicity effect	HeLa	(Cheng et al., 2020; Shabir et al., 2018)
Pleurocidin	*Pleuronectes americanus*	Cytotoxicity effect	NRC-03 and NRC-07	(Deslouches & Di, 2017; Shabir et al., 2018)
Piscidin	*Morone saxatilis*	Cytotoxicity effect	CRL-11226	(Deslouches & Di, 2017)
Cecropin A Ceropin B	*Pleuronectes americanus*	Membrane perturbation and apoptosis	HTC and STC	(Deslouches & Di, 2017)
CA-MA-2	*Hybrid fish*	Membrane perturbation	STC-1	(Kunda, 2020)
MG2B	*Hybrid fish*	Membrane perturbation	MCF-7	(Lugo et al., 2019)
TP4	*Oreochromis niloticus*	Cytotoxicity effect	U87MG and U251	(Kang et al., 2018)
PACAP	*Clarias gariepinus*	Cytotoxicity effect	H460	(Wang et al., 2017)
YALRAH	*Setipinna taty*	Antiproliferative activity	PC-3	(Shabir et al., 2018; Wang et al., 2017)
MSP-4	*Oreochromis niloticus*	Induce apoptosis	MG63	(Deslouches & Di, 2017; Wang et al., 2017)
Histone H2A	*Fenneropenaeus indicus*	Cytotoxicity effect	NCI–H460 and HEp-2	(Deslouches & Di, 2017; Kang et al., 2018; Shabir et al., 2018; Wang et al., 2017)

isolated from mucus and skin of different fish such as *Red sea bream*, *Salmo salar*, and *Pardachirus marmoratus* (Deslouches & Di, 2017; Kunda, 2020; Marqus et al., 2017; Nurdiani et al., 2016; Wang et al., 2018). Pardaxin A inhibits various cancer cell lines, including HT1080T, Hela, HP-1, and U937, through membrane perturbation, apoptosis, inducing maturation and differentiation of cells (Cheng et al., 2020; Kang et al., 2018). Another common peptide was Epinecidin-1 which also showed anticancer activity, as reported in four previous studies (Deslouches & Di, 2017; Kang et al., 2018; Marqus et al., 2017). Epinecidin-1 isolated from *Gadus morhua*, *Epinephelinae, Pleuronectes platessa*, and *Epinephelus coioides* have been reported to induce cancer cell death (e.g., HTC and STC) by membrane lysis and necrosis (Varier et al., 2018; Wang et al., 2017). Other AMP, namely TP4 isolated from *Hyalophora cecropia* and *Oreochromis niloticus* fish, have been reported to have a cytotoxicity effect against U87MG and U251 cell lines (Cheng et al., 2020; Shabir et al., 2018). Other AMPs such as Chrysophsin-1 A (Wang et al., 2017; Deslouches et al., 2018), Piscidin (Cheng et al., 2020; Shabir et al., 2018), and Cecropin A and B (Shabir et al., 2018) were mentioned in two studies, where each showed cytotoxic effect against different cancer cell lines such as HeLa, A549, CRL-11226, HTC, and STC through membrane perturbation and apoptosis (Deslouches & Di, 2017; Shabir et al., 2018). Piscidin-1 derived from *Morone saxatilis* has been reported to have anti-proliferation and anticancer properties as well as prevent bacterial infections (Shabir et al., 2018). These AMPs get attention as anticancer because they can specifically kill cancer cells without harming healthy cells (Kunda, 2020).

However, there are other 14 AMPs were found in this systematic review, including TFD100 (Hansen et al., 2020), Turgencin A, B (Hansen et al., 2020), TH1-5, TH2-2, 3, 5 (Wang et al., 2017), Pleurocidin (Shabir et al., 2018), CA-MA-2, MG2B (Deslouches & Di, 2017), PACAP (Lugo et al., 2019), YALRAH (Kang et al., 2018), MSP-4 (Kuo et al., 2018), Histone H2A (Sruthy et al., 2019). These peptides were isolated from a wide range of fish organs and showed the ability to inhibit the growth of different cancer cell lines such as HUVECs, HeLa (Wang et al., 2017), A2058, MRC-5 (Hansen et al., 2020), NRC-03, NRC-07 (Shabir et al., 2018), STC-1, MCF-7 (Deslouches & Di, 2017), H460 (Lugo et al., 2019), PC-3 (Kang et al., 2018), MG63 (Kuo et al., 2018), NCI–H460, and HEp-2 (Sruthy et al., 2019) (Table 2).

Some AMPs derived from fish have novel pathways for the application of antitumor therapy (Cheng et al., 2020; Shabir et al., 2018). Pleurocidin, which is derived from the *Pleuronectes americans* and members of this cationic peptide family (NRC-3 and NRC), has been reported to be cytotoxic against human breast cancer cells and mammalian carcinoma cells but not in human skin fibroblasts (Cheng et al., 2020; Shabir et al., 2018). PACAP of *Clarias gariepinus* fish also has been reported to show activity against human lung cancer cell line, H460 (Lugo et al., 2019). Furthermore, it has been observed that

AMP isolated from Aluterus monoceros inhibited the viability of COLO320 cancer cells (Neshani et al., 2019). Although there are many reports on the ability of AMP from fish to act as an anticancer, the physicochemical parameters that define some of the activity of AMP on cancer cells are still unknown (Neshani et al., 2019).

This systematic review has identified 38 AMPs with antimicrobial activity (Table 3). Epinecidin-1, Pardaxin, and Piscidin 1, 2, 3 were the most reviewed peptides that have antimicrobial properties (4 studies each) (Deslouches & Di, 2017; Kang et al., 2018; Marqus et al., 2017), followed by Pleurocidin, Cathelicidin 1, and Cathelicidin 2 (3 studies each). Two studies were reported for TP4, β-defensin, Piscidin, Hepcidin, Histone-derived, Moronecidin, Chrysophsin-1 A, TH1-5, Grammistins, HPL-1, and HPL-2. Other peptides like Turgencin A, B, PACAP, Epinecidin, Hipposin, Misgurin, Oncorhyncin II, TFD100, TH2-2, 3, HFIAP-1, 2, 3, Orpotrin, HbβP-1, 2, 3, Pelteobagrin, and TnP were mentioned in only one study each. These peptides were derived from a wide range of fish, as shown in Table 3.

Table 3
Summary of the reported antimicrobial peptides extracted from fish

Antimicrobial Peptide	Source	References
Pleurocidin	*Pleuronectes americanus*	(Shabir et al., 2018; Valero et al., 2020)
Turgencin A	*ascidian Synoicum*	(Lugo et al., 2019)
Turgencin B	*ascidian Synoicum*	(Shabir et al., 2018; Cipolari et al., 2020)
TP4	*Oreochromis niloticus*	(Shabir et al., 2018); Bo et al., 2019)
PACAP	*Clarias gariepinus*	(Shabir et al., 2018)
Cathelicidin 1 Cathelicidin 2	*Oncorhynchus mykiss* and *Salmo salar*	(Bo et al., 2019)
β-defensin	*Danio rerio*	(Shabir et al., 2018)
Piscidin	*Morone chrysops*	(Shabir et al., 2018)
Hepcidin	*Channa maculate* and *Sebastiscus marmoratus*	(Shabir et al., 2018)
Epinecidin	*Epinephelus coioides*	(Shabir et al., 2018; Valero et al., 2020)
Hipposin	*Hippoglossus hippoglosus*	(Shabir et al., 2018)
Histone-derived	*Salmo salar* and *Fenneropenaeus indicus*	(Shabir et al., 2018; Wang et al., 2017; Cipolari et al., 2020)
Moronecidin	*Morone chrysops*	(Shabir et al., 2018)
Misgurin	*Misgurnus*	(Wang et al., 2017)
Pardaxin	*Pardachirus and Salmo salar*	(Valero et al., 2020; Chaturvedi et al., 2020; Chee et al., 2019)
Oncorhyncin II	*Onchorhynchus mykiss*	(Wang et al., 2017; Valero et al., 2020)
TFD100	Codfish and Blue whiting	(Wang et al., 2017; Valero et al. 2020)
Epinecidin-1	Codfish, Plaice, Groupers, and *Epinephelus coioides*	(Wang et al., 2017)

Table 3 *(continue)*

Antimicrobial Peptide	Source	References
Chrysophsin-1 A	Red sea bream	(W et al., 2017)
TH1-5	*Oreochromis niloticus*	(Cipolari et al., 2020; Valero et al., 2020)
TH2-2	*Oreochromis niloticus*	(Chaturvedi et al., 2020)
TH2-3	*Oreochromis niloticus*	(Cipolari et al., 2020)
Grammistins	*Grammistes sexlineatus* and *Pogonoperca punctata*	(Cipolari et al., 2020)
Piscidin 1 Piscidin 2 Piscidin 3	*Morone saxatilis* and *M. chrysops* *Sebastiscus marmoratus*	(Cipolari et al., 2020)
HFIAP-1 HFIAP -2 HFIAP -3	*Myxine glutinosa*	(Cipolari et al., 2020)
HPL-1 HPL-2	*Ictalurus punctatus, M. saxatilis,* and *Oncorhyncus mykiss.*	(Cipolari et al., 2020)
Orpotrin	Potamotrygon	(Cipolari et al., 2020)
TnP	*Thalassophyte nattereri*	(Shabir et al., 2018; Cipolari et al., 2020; Chaturvedi et al., 2020)

Hepcidin 1, Piscidin, Moronecidin, and β-defensin AMPs isolated from *Sebastiscus marmoratus* liver and *Danio rerio* act as a multilevel network of antimicrobial defensive and has the potential to be used to fight pathogenic infections in aquaculture (Bo et al., 2019). Although PACAP is a regulatory neuropeptide that belongs to the secretin/glucagon superfamily, it showed antimicrobial activity against 43 pathogenic microorganisms (Lugo et al., 2019). While for Epinecidin-1 derived from the *Epinephelus coioides*, which consist of 21 amino acids, from the amino acid sequence of 22–42 residues of Epi-1—GFIFHIIKGLFHAGKMIHGLV (Chee et al., 2019), it demonstrated diverse pharmacological activities besides antimicrobial including immunomodulatory and wound healing properties (Chee et al., 2019). As AMPs exhibit a broad range of antimicrobial activity against viruses, fungi, and bacteria, fish-derived AMPs might be a possible candidate for future aquaculture, medical, or host protection modulation applications in water (Valero et al., 2020).

Other Activity of Antimicrobial Peptides

In addition to having antimicrobial and anticancer properties, AMPs is also reported to have other functional features such as immunomodulatory (five studies) (Abuine et al., 2019; Chee et al., 2019; Cipolari et al., 2020), a potent antioxidant (three studies) (Abuine et al., 2019; Chee et al., 2019), active antihypertensive function (Chee et al., 2019), hemolytic activity (Buonocore et al., 2019; Kumar et al., 2019), wound healing (Chee et al., 2019;

Neshani et al., 2019) (two studies each), and antidiabetic (Neshani et al., 2019), anti-inflammation (Neshani et al., 2019), and anti-Alzheimer's (Abuine et al., 2019) (one study each). The collagen hydrolysates of by-products fish, including giant squid (*Dosidicus Gigas*) Croceine croaker scale (*Pseudosciaena crocea*), and Spanish mackerel skin (*Scomberomorous niphonius*), are pertained to have anti-oxidative and antihypertensive properties (Felício et al., 2017). The Black-barred halfbeak *(Hemiramphus far*) gelatin extract skin also showed an antioxidant function (Abuine et al., 2019). *Cobia* skin protein and *Raja clavata* skin lower molecular weight hydrolysates showed greater antioxidant capacity than Nile tilapia skin gelatin (Abuine et al., 2019).

The reviewed studies also reported many AMPs that can prevent a wide range of virus infections, including those caused by enveloped viruses (RNA, DNA, feline calicivirus, and echovirus) (Abuine et al., 2019; Chee et al., 2019; Cipolari et al., 2020). Some alpha-helical peptides have shown strong anti-HSV action, in addition to magainins, dermaseptin, and melittine. β-sheet peptides for example the β-turn peptide lactoferricin, defensin, tachyplesin, and protegrin, were also observed to have significant activity against HSV (Wang et al., 2018).

Membrane Differences Between Bacterial and Cancer Cells

Some of the reviewed studies indicated physicochemical differences in the membrane between bacterial and cancer cells (Abuine et al., 2019; Buonocore et al., 2019; Chee et al., 2019; Kumar et al., 2019). The physicochemical parameters which define some AMPs' activity against cancer cells are still unknown (Chee et al., 2019; Neshani et al., 2019). Efforts of existing research have been made to consider these discrepancies, which will make for better production of AMPs. Certain AMPs can be used as anticancer irrespective of the recognition source or synthetic design pathway, whereby the physicochemical properties of these peptides paved the way for the behavior of the pathogen (Buonocore et al., 2019; Chee et al., 2019; Kumar et al., 2019; Neshani et al., 2019). However, the evidence recognized that not all AMPs have anticancer activity (Felício et al., 2017; Wang et al., 2018).

Mechanisms of AMPs Action

Reviewed evidence then identified many primary mechanisms of AMPs' action, but the precise process by which bioactive peptides destroy cancer cells is still unclear (Abuine et al., 2019; Chee et al., 2019; Buonocore et al., 2019; Kumar et al., 2019). Wang et al. (2017) suggested that the anticancer activity of bioactive peptides can usually be mediated either by membranolytic or non-membranolytic mechanisms (Wang et al., 2017). Boparai and Sharma (2019) mentioned that AMPs could interact with bacterial cell membranes through electrostatic interactions, thus making it difficult for bacteria to develop resistance, unlike conventional antibiotics.

Membrane-Related Mechanisms

The mammalian cell membranes possess a bilayer of phospholipids comprising both hydrophilic and hydrophobic regions. Net electron-charged proteins also exist on the inside of the cell membrane. Some evidence indicates that AMPs kill cancer cells by destroying the cell membrane (Boparai & Sharma, 2019; Wang et al., 2017). In particular, peptides target elements of the membrane that are negatively charged, such as phosphatidylserine (PS), sialic acid, or heparin sulfate (Boparai & Sharma, 2019; Wang et al., 2017). However, other sources of negatively charged molecules should also be considered when considering the activity and efficacy of AMPs. These sources of negatively charged molecules are often over-expressed on plasma membranes other than cancer, such as sialylated glycoproteins or proteoglycans with heparin sulfate (Felício et al., 2017). An essential characteristic in the cytotoxicity induced by AMPs on cancer cells is the electrostatic interactions between cationic AMPs and the cancer cell membrane (Chee et al., 2019; Felício et al., 2017; Neshani et al., 2019).

Pardaxin from the sole of the Red Sea are examples of AMPs that cause cell lysis by creating pores and adopting electrostatic interactions (Chee et al., 2019; Felício et al., 2017). They are owing to the broad arrangement of AMPs on the membrane surface; this aggregation mechanism may also cause membrane depolarization or create toroidal-shaped transmembrane pores (Chee et al., 2019). Post-translation changes such as glycosylation of membrane-associated glycoproteins in cancer cells make them negatively affected more than normal cells (Kunda, 2020; Shabir et al., 2018). The mechanism underlying each peptide's membranolytic activity depends on the characteristics of the bioactive peptide and those of the target membrane, which in effect modulates the selectivity and toxicity of the peptides (Kunda, 2020). Bioactive peptide-induced membrane disruption can occur in different ways, including lipid pores (barrel-stave and toroidal pore models), membrane bilayer thinning, membrane dissolution (carpet model), or lipid-peptide domain formation (Figure 2) described by Wang et al. (2017).

Non-Membrane Related Mechanisms

Non-membrane mode refers to mitochondrial membrane disruptions or mitochondrial-dependent apoptosis, a condition in which cell death is caused by disrupting the plasma membranes. This process plays a crucial role in carcinogenesis and cancer therapy, as shown in Figure 3 (Cheng et al., 2020: Wang et al., 2017). AMPs primarily work on target cell membranes through a non-receptor-mediated pathway (Valero et al., 2020). An example of AMP involving non-membrane mechanisms is Pleurocidin derived from *Pleuronectes Americans*. The members of this cationic peptide family (NRC-3 and NRC) have been shown to disrupt the stability of the cell membrane (Valero et al., 2020). Meanwhile, the antitumor process of B1 peptide and its analogs includes three steps, first, disturbance of

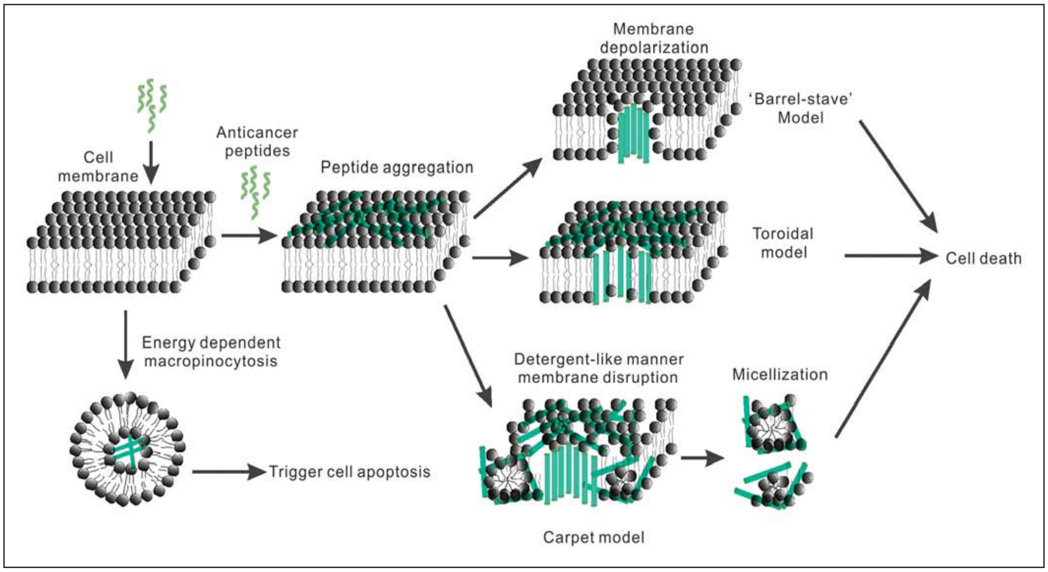

Figure 2. Schematic illustration of cell entry mechanisms of anticancer and antimicrobial peptides (Adapted from Wang et al., 2017)

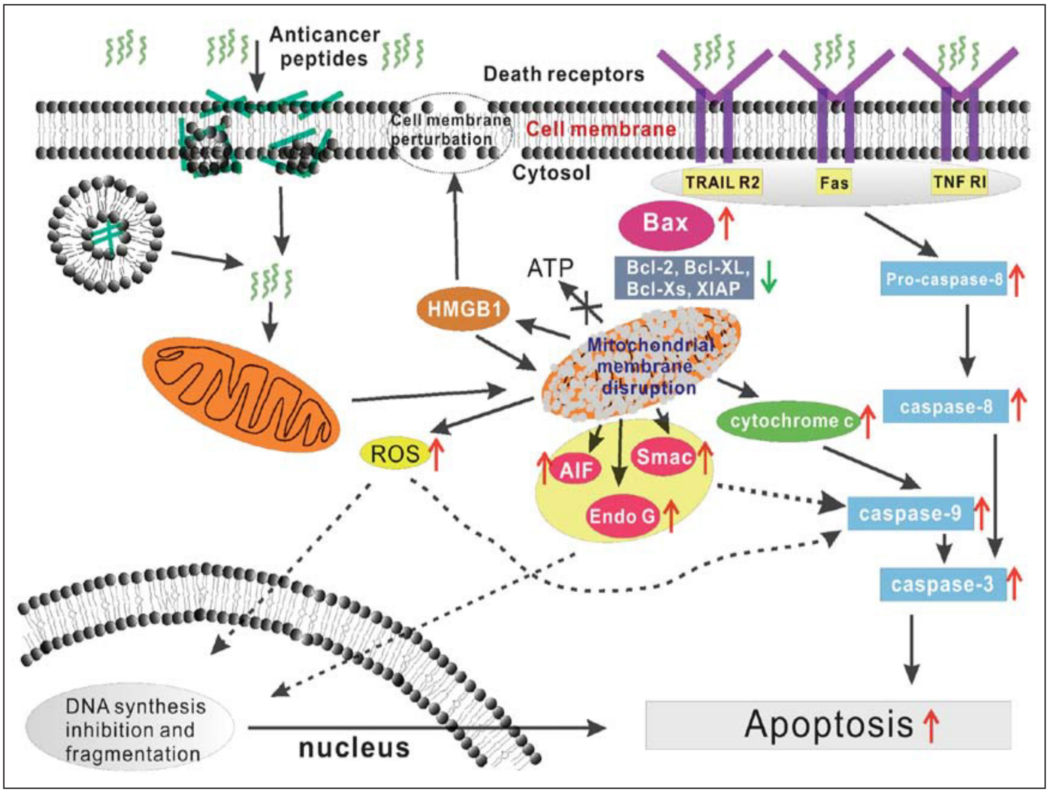

Figure 3. Mechanisms of action of anticancer and antimicrobial peptides via non-membrane related mechanisms pathways (Adapted from Wang et al., 2017)

the cell membrane resulting from changes in membrane permeability; second, cytoplasm penetration after membrane disruption; third, disruption of mitochondrial membranes and release of cytochrome C (Valero et al., 2020). Two specific peptides in tuna fish were also extracted from the dark muscle and found to be antiproliferative, indicating breast cancer cell development inhibition (MCD-7 cell lineage) (Wang et al., 2018). AMPs derived from marine organisms could cause tumor cell death, which uses apoptosis to destroy tumor cells during therapy (Wang et al., 2018).

Limitation

Evidence from *in-vitro* studies suggested that AMPs have the potential to be used in the treatment of various diseases such as cancer, where they were able to inhibit various types of cancer cells such as breast, lung, leukemia, bone, and so on (Chee et al., 2019; Kumar et al., 2019; Felício et al., 2017; Neshani et al., 2019). The physicochemical characteristics of these AMPs have shown a strong effect against different cancer cell lines (Felício et al., 2017). These AMP compounds act as anticancer by neutralizing free radicals, triggering apoptosis, and inhibiting angiogenesis (Chee et al., 2019; Felício et al., 2017). However, the mechanisms of AMPs' action *in-vivo* against these cells are still unknown. The use of AMP as an anticancer was based on a synthetic design process. Besides their antimicrobial and anticancer properties, the reviewed AMPs were also able to exhibit different therapeutics properties such as immunomodulatory, active antihypertensive function, a potent antioxidant, hemolytic activity, wound healing, anti-inflammation, antidiabetic, and anti-Alzheimer's (Buonocore et al., 2019; Chee et al., 2019; Kumar et al., 2019; Neshani et al., 2019).

The broad spectrum of antimicrobial activity and non-specific functions of AMPs illustrate their potential for use as an alternative to antibiotics (Wang et al., 2018). AMPs of various species of fish have demonstrated a strong effect against cancer cell lines and different human pathogens. Evidence from *in-vitro* and *in-vivo* studies suggested that AMPs peptides have the potential to be used in the treatment of various diseases, including cancer. The physicochemical characteristics of these AMPs have shown a strong effect against different cancer cell lines (Felício et al., 2017). The mechanisms of AMPs' action *in-vivo* against these cells are still unknown. The use of AMP as an anticancer is based on a synthetic design process. Despite having a variety of benefits, AMPs have several disadvantages, including a lack of oral bioavailability and poor physiological stability. Besides, gastric acid and complex enzymes present in the gastrointestinal system cause EMP to degrade (Wang et al., 2017).

The limitations found in this systematic review were the inclusion of papers written in English and published between 2017 and 2020. The authors excluded the investigations written in other languages published before 2017. In future studies, medium to long-term randomized clinical trials is needed to confirm the effect of peptides on the disease and test their effectiveness in increasing resistance to chronic disease. Therefore, further studies must be continued to link the advantages of AMP, its peptidomimetics, and antibiotics to reduce drug resistance (Cipolari et al., 2020; Wang et al., 2018). There is also a need for further studies on natural AMPs, especially those obtained from fish, to test their ability to cure human cancer and chronic diseases. The use of AMPs from fish has been a potential therapeutic strategy to be applied in the future (Chee et al., 2019; Felício et al., 2017). Further and continuous study in the synthesis and characterization of these peptides is very important to ensure their effectiveness and usability. AMPs from fish have been a promising source of therapeutic strategies. Further, to enhance the activity of these peptides need to develop and synthesized.

CONCLUSION

As far as the authors are aware, this is the first systematic review conducted on the recent AMPs isolated from fish, focusing on the antimicrobial and anticancer activity. The findings of this systematic review revealed that there are 38 AMPs isolated from various fish species (n= 49) that showed potential activity against medically important pathogens in humans and fish. Twenty-one AMPs showed a significant anticancer activity besides their antimicrobial activity. The most common AMP was the Pardaxin, which exhibits anticancer, antimicrobial, antifungal, antiviral, immunomodulatory, and antioxidant activities (Cipolari et al., 2020; Deslouches & Di, 2017; Uen et al., 2019; Wang et al., 2018). Epinecidin-1, TP4, and Piscidin were also common peptides in the reviewed studies that showed anticancer and antimicrobial activities (Deslouches & Di, 2017; Uen et al., 2019; Wang et al., 2018). The broad spectrum of antimicrobial activity and non-specific functions of such AMPs illustrate their potential for use as an alternative to antibiotics (Wang et al., 2018).

The presence of adverse effects from anticancer drugs and the formation of drug resistance in cancer cells makes it necessary to study natural resources to produce healthier and more effective drugs than existing products. This study provides a broad overview of the recent AMPs isolated from various fish species that showed promising therapeutic activities. Current AMPs therapy is effective in inhibiting various bacteria. In addition, it also shows the potential ability to treat cancer cells, including the breast, colon, lungs, thyroid, pancreas, and so on (Boparai & Sharma, 2019; Cipolari et al., 2020; Kumar et al., 2019; Neshani et al., 2019; Wang et al., 2018). The findings of this systematic review indicate that AMP found in fish has the potential to be used in pharmacology, especially anticancer therapy. This review successfully shows a specific direction for future projects utilizing natural products, namely fish AMP, to provide new drugs in treating cancer.

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REFERENCES

Abuine, R., Rathnayake, A. U., & Byun, H. G. (2019). Biological activity of peptides purified from fish skin hydrolysates. *Fisheries and Aquatic Sciences*, *22*(1), 1-14. https://doi.org/10.1186/s41240-019-0125-4

Acosta, J., Roa, F., González-Chavarría, I., Astuya, A., Maura, R., Montesino, R., Muñoz, C., Camacho, F., Saavedra, P., Valenzuela, A., Sánchez, O., & Toledo, J. R. (2019). *In vitro* immunomodulatory activities of peptides derived from Salmo salar NK-lysin and cathelicidin in fish cells. *Fish and Shellfish Immunology, 88*, 587-594. https://doi.org/10.1016/j.fsi.2019.03.034

Anju, A., Smitha, C. K., Preetha, K., Boobal, R., & Rosamma, P. (2019). Molecular characterization, recombinant expression and bioactivity profile of an antimicrobial peptide, Ss-arasin from the Indian mud crab, Scylla serrata. *Fish and Shellfish Immunology, 88*, 352-358. https://doi.org/10.1016/j.fsi.2019.03.007

Ardeshir, R. A., Rastgar, S., Morakabati, P., Mojiri-Forushani, H., Movahedinia, A., & Salati, A. P. (2020). Selective induced apoptosis and cell cycle arrest in MCF7 and LNCap cell lines by skin mucus from round goby (Neogobius melanostomus) and common carp (Cyprinus carpio) through P53 expression. *Cytotechnology, 72*(3), 367-376. https://doi.org/10.1007/s10616-020-00383-x

Bhandari, D., & Rafiq, S., Gat, Y., Gat, P., Waghmare, R., & Kumar, V. (2020). A review on bioactive peptides: Physiological functions, bioavailability and safety. *International Journal of Peptide Research and Therapeutics, 26,* 139-150. https://doi.org/10.1007/s10989-019-09823-5

Bo, J., Yang, Y., Zheng, R., Fang, C., Jiang, Y., Liu, J., Chen, M., Hong, F., Bailey, C., Segner, H., & Wang, K. (2019). Antimicrobial activity and mechanisms of multiple antimicrobial peptides isolated from rockfish *Sebastiscus marmoratus*. *Fish and Shellfish Immunology, 93*, 1007-1017. https://doi.org/10.1016/j.fsi.2019.08.054

Boparai, J. K., & Sharma, P. K. (2019). Mini review on antimicrobial peptides, sources, mechanism and recent applications. *Protein Peptide Letter*, *27*(1), 4-16. https//doi.org/ 10.2174/0929866526666190822165812

Buonocore, F., Picchietti, S., Porcelli, F., Pelle, G. D., Olivieri, C., Poerio, E., Bugli, F., Menchinelli, G., Snaguinetti, M., Bresciani, A., Gannari, N., Taddei, A. R., Fausto, A. M., & Scapiliati, G. (2019). Fish-derived antimicrobial peptides: Activity of a chionodracine mutant against bacterial models and human bacterial pathogens. *Developmental & Comparative Immunology*, *96*, 9-17. https://doi.org/10.1016/j.dci.2019.02.012

Chang, S. M., Matchar, D. B., Smetana, G. W., & Umscheid, C. A. (Eds.). (2012). *Methods guide for medical test reviews*. Agency for Healthcare Research and Quality (US).

Chaturvedi, P., Bhat, R. A. H., & Pande, A. (2020). Antimicrobial peptides of fish: Innocuous alternatives to antibiotics. *Reviews in Aquaculture*, *12*(1), 85-106. https://doi.org/10.1111/raq.12306

Chee, P. Y., Mang, M., Lau, E. S., Tan, L. T., He, Y. W., Lee, W. L., Pusparajah, P., Chan, K. G., Lee, L. H., & Goh, B. H. (2019). Epinecidin-1, an antimicrobial peptide derived from Grouper (*Epinephelus coioides*):

Pharmacological activities and applications. *Frontiers In Microbiology, 10*, Article 2631. https://doi.org/10.3389/fmicb.2019.02631

Cheng, M. H., Pan, C. Y., Chen, N. F., Yang, S. N., Hsieh, S., Wen, Z. H., Chen, W. F., Wang, J. W., Lu, W. H., & Kuo, H. M. (2020). Piscidin-1 induces apoptosis via mitochondrial reactive oxygen species-regulated mitochondrial dysfunction in human osteosarcoma cells. *Scientific Reports, 10*(1), Article 5045. https://doi.org/10.1038/s41598-020-61876-5

Cipolari, O. C., de Oliveira Neto, X. A., & Conceição, K. (2020) Fish bioactive peptides: A systematic review focused on sting and skin. *Aquaculture*, *515*, Article 734598. https://doi.org/10.1016/j.aquaculture.2019.734598

Deslouches, B., & Di, Y. P. (2017). Antimicrobial peptides with selective antitumor mechanisms: Prospect for anticancer applications. *Oncotarget*, *8*(28), 46635-46651. https://doi.org/10.18632/oncotarget.16743

Devi, N. P., Das, S. K., Sanjukta, R. K., & Singh, S. G. (2019). A comparative study on antibacterial activity of integumentary extract of selected freshwater fish species and neem extracts against gram-positive and gram-negative bacteria. *Journal of Entomology and Zoology Studies*, *7*(2), 1352-1355.

Felício, M. R., Silva, O. N., Gonçalves, S., Santos, N. C., & Franco, O. L. (2017). Peptides with dual antimicrobial and anticancer activities. *Frontiers in Chemistry*, *5*(5), 1-9. https//doi.org/10.3389/fchem.2017.00005

Hansen, I., Isaksson, J., Poth, A. G., Hansen, K. Ø., Andersen, A., Richard, C., Blencke, H. M., Stensvåg, K., Craik, D. J., & Haug, T. (2020). Isolation and characterization of antimicrobial peptides with unusual disulfide connectivity from the colonial ascidian *Synoicum turgens*. *Marine Drugs, 18*(1), Article 51. https://doi.org/10.3390/md18010051

Hazam, P. K., & Chen. J. H. (2020). Therapeutic utility of the antimicrobial peptide Tilapia Piscidin 4 (TP4). *Aquaculture Reports 17*, Article 100409. https://doi.org/10.1016/j.aqrep.2020.100409

Kang, H. K., Choi, M. C., Seo, C. H., & Park, Y. (2018). Therapeutic properties and biological benefits of marine-derived anticancer peptides. *International Journal of Molecular Sciences, 19*(3), Article 919. https://doi.org/10.3390/ijms19030919

Kumar, P., Rajeshwaran, T., Priya, P., Kailasam, M., Biswas, G., Ghosal, T. K., Vijayan, K. K., & Arasu, A. R. T. (2019). Comparative immunological and biochemical properties of the epidermal mucus from three brackishwater fishes. *Proceedings of the National Academy of Sciences, India Section B: Biological*, *89*(1), 95-103. https://doi.org/10.1007/s40011-017-0923-3

Kunda N. K. (2020). Antimicrobial peptides as novel therapeutics for non-small cell lung cancer. *Drug Discovery Today, 25*(1), 238-247. https://doi.org/10.1016/j.drudis.2019.11.012

Kuo, H. M., Tseng, C. C., Chen, N. F., Tai, M. H., Hung, H. C., Feng, C. W., Cheng, S. Y., Huang, S. Y., Jean, Y. H., & Wen, Z. H. (2018). MSP-4, an antimicrobial peptide, induces apoptosis via activation of extrinsic Fas/FasL- and intrinsic mitochondria-mediated pathways in one osteosarcoma cell line. *Marine Drugs, 16*(1), Article 8. https://doi.org/10.3390/md16010008

León, R., Ruiz, M., Valero, Y., Cardenas, F., Guzman, F., Vila, M., & Cuesta, A. (2020). Exploring small cationic peptides of different origin as potential antimicrobial agents in aquaculture. *Fish Shellfish Immunollogy*, *98*, 720-727. https//doi.org/10.1016/j.fsi.2019.11.019

Lirio, G. A. C., De Leon, J. A. A., & Villafuerte, A. G. (2019). Antimicrobial activity of epidermal mucus from top aquaculture fish species against medically-important pathogens. *Walailak Journal of Science and Technology, 16*(5), 329-340. https://doi.org/10.48048/wjst.2019.6287

Lugo, J. M., Tafalla, C., Oliva, A., Pons, T., Oliva, B., Aquilino, C., Morales, R., & Estrada, M. P. (2019). Evidence for antimicrobial and anticancer activity of pituitary adenylate cyclase-activating polypeptide (PACAP) from North African catfish (Clarias gariepinus): Its potential use as novel therapeutic agent in fish and humans. *Fish Shellfish Immunology, 86*, 559-570. https://doi.org/10.1016/j.fsi.2018.11.056

Marqus, S., Pirogova, E., & Piva, T. J. (2017). Evaluation of the use of therapeutic peptides for cancer treatment. *Journal of Biomedical Sciences*, *24*(1), 1-15. https://doi.org/10.1186/s12929-017-0328-x

Moher, D., Liberati, A., Tetzlaff, J., & Altman, D. G. (2009). Preferred reporting items for systematic reviews and meta-analyses: The PRISMA statement. *BMJ, 339,* Article b2535. https://doi.org/10.1136/bmj.b2535

Munn, Z., Moola, S., Lisy, K., Riitano, D., & Tufanaru, C. (2015). Methodological guidance for systematic reviews of observational epidemiological studies reporting prevalence and cumulative incidence data. *International Journal of Evidence-based Healthcare, 13*(3), 147-153. https://doi.org/10.1097/XEB.0000000000000054

Neshani, A., Zare, H., Eidgahi, M. R. A., Chichaklu, A. H., Movaqar, A., & Ghazvini, K. (2019). Review of antimicrobial peptides with anti-Helicobacter pylori activity. *Helicobacter, 24(*1), Article e12555. http://dx.doi.org/10.1111/hel.12555

Nurdiani, R., Dissanayake, M., Street, W. E., Donkor, O. N., Singh, T. K., &. Vasiljevic, T. (2016). *In vitro* study of selected physiological and physicochemical properties of fish protein hydrolysates from 4 Australian fish species. *International Food Research Journal, 23*(5), 2029-2040.

Offret, C., Jégou, C., Mounier, J., Fleury, Y., & Le Chevalier, P. (2019). New insights into the haemo- and coelo-microbiota with antimicrobial activities from Echinodermata and Mollusca. *Journal of Applied Microbiology, 126*(4), 1023-1031. https://doi.org/10.1111/jam.14184

Pan, C. Y., Tsai, T. Y., Su, B. C., Hui, C. F., & Chen, J. Y. (2017). Study of the antimicrobial activity of Tilapia Piscidin 3 (TP3) and TP4 and their effects on immune functions in hybrid Tilapia (*Oreochromis* spp.). *PloS One, 12*(1), Article e0169678. https://doi.org/10.1371/journal.pone.0169678

Raju, V. S., Sarkar, P., Pachaiappan, R., Paray, B. A., Al-Sadoon, M. K., & Arockiaraj, J. (2020). Defense involvement of piscidin from striped murrel *Channa striatus* and its peptides CsRG12 and CsLC11 involvement in an antimicrobial and antibiofilm activity. *Fish and Shellfish Immunology, 99,* 368-378. https://doi.org/10.1016/j.fsi.2020.02.027

Russell, C. K., & Gregory, D. M. (2003). Evaluation of qualitative research studies. *Evidence-Based Nursing, 6*(2), 36-40. http://dx.doi.org/10.1136/ebn.6.2.36

Shabir, U., Ali, S., Magray, A. R., Ganai, B. A., Firdous, P., Hassan, T., & Nazir, R. (2018). Fish antimicrobial peptides (AMP's) as essential and promising molecular therapeutic agents: A review. *Microbial Pathogenesis, 114*, 50-56. https://doi.org/10.1016/j.micpath.2017.11.039

Sruthy, K. S., Nair, A., Antony, S. P., Puthumana, J., Singh, I., & Philip, R. (2019). A histone H2A derived antimicrobial peptide, Fi-Histin from the Indian white shrimp, *Fenneropenaeus indicus*: Molecular and functional characterization. *Fish and Shellfish Immunology, 92*, 667-679. https://doi.org/10.1016/j.fsi.2019.06.044

Uen, W. C., Choong, C. Y., Tai, C. J., & Tai, C. J. (2019). Pardaxin promoted differentiation and maturation of leukemic cells via regulating TLR2/MyD88 signal against cell proliferation. *Evidence-based Complementary and Alternative Medicine, 2019*, Article 7035087. https://doi.org/10.1155/2019/7035087

Valero, Y., Saraiva-Fraga, M., Costas, B., & Guardiola, F. A. (2020). Antimicrobial peptides from fish: Beyond the fight against pathogens. *Reviews in Aquaculture*, *12*, 224-253. https://doi.org/10.1111/raq.12314

Varier, K. M., Chinnasamy, A., Gajendran, B., & Nagarathnam, R. (2018). Isolation and characterization of a novel anticancer muscle protein from edible marine catfish tachysurus dussumeiri. *International Journal of Pharmaceutical Sciences and Research, 9*(7), 2720-2730. https://doi.org/10.13040/IJPSR.0975-8232.9(7).2720-30

Walsh, D., & Downe, S. (2006). Appraising the quality of qualitative research. *Midwifery, 22*(2), 108-119. https://doi.org/10.1016/j.midw.2005.05.004

Wang, J., Dou, X., Song, J., Lyu, Y., Zhu, X., Li, W., & Shan, A. (2018). Antimicrobial peptides: Promising alternatives in the post feeding antibiotic era. *Medicinal Research Reviews, 39*(3), 831-859. https://doi.org/10.1002/med.21542

Wang, X., Yu, H., Xing, R., & Li, P. (2017). Characterization, preparation, and purification of marine bioactive peptides. *Biomed Research International*, *2017*, Article 9746720. https://doi.org/10.1155/2017/9746720