Antibacterial Properties of Antimicrobial peptides (AMP) from Skin Extracts of *Catla catla* against Wound Pathogens

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Abstract: *Wound infection is a major complication in healing of chronic wounds. Diabetic wounds are highly prone to wound infection and development of drug resistance among pathogens has created a necessity for development of new antimicrobials for treatment of such wound infections. Antimicrobial peptides (AMP) are found to be potent source showing promising antimicrobial action. Therefore the present study focuses on extraction of AMP from Catla catla skin using two different solvents and determining antibacterial activity against wound pathogens. Specimens from wound were collected using sterile swabs and streaked on nutrient agar. Then the colonies were subjected to gram staining and series of biochemical tests for identification. Live fish of Catla catla weighing 470gm was collected, homogenized in two different solvents (Ethanol and water). Antibacterial activity of the crude extracts of AMP was evaluated by using well diffusion method. Based on the colony morphology and biochemical tests the pathogens were found to be Staphylococcus aureus and Pseudomonas aeruginosa. The wound pathogens isolated were also found to be a biofilm producer. Antibacterial analysis showed ethanolic extract had higher inhibition than aqueous extracts. Therefore the extracted AMP can be used for development of novel antibacterial drugs for treatment of drug resistant bacterial infections.*

Keywords: Antimicrobial peptides (AMP), *Catla catla*, Wound infection, wound pathogen, Antibacterial analysis

I. Introduction

Wound infection is arguably the most common, and at the same times the most potentially devastating, complication of the wound healing process. When inadequately managed, wound infection can incur increased medical expenses, lead to secondary complications, and even cause loss of limb or life. For these reasons, a number of treatment methods were practiced in order to cure wound infections [1]. The usual pathogens on skin infections are *Staphylococcus aureus, Streptococcus pyogenes, Enterococci* and *Pseudomonas aerugionsa.* Most infected wounds are caused by bacterial colonization, originating either from the normal flora on the skin, or bacteria from other parts of the body or the outside environment [2, 3].

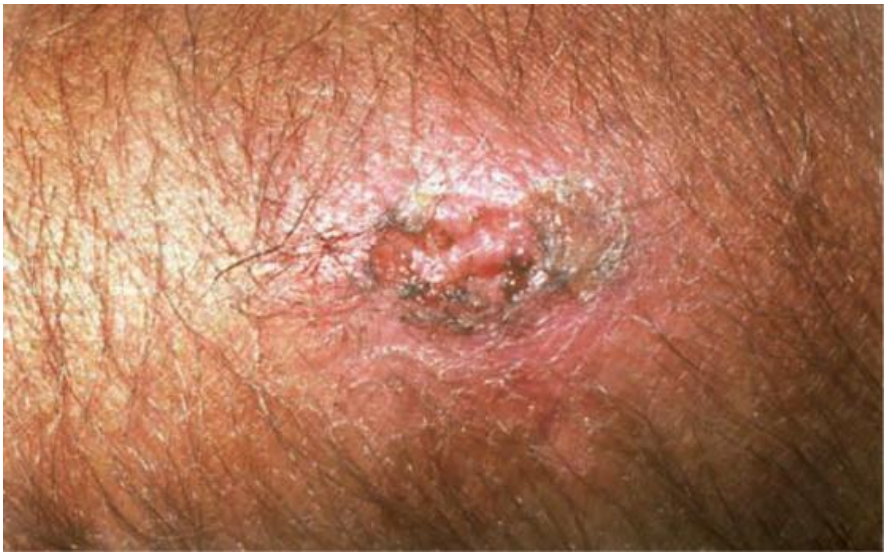
Now-a-days the development of resistance by a pathogen to many of the commonly used antibiotics provides an impetus for further attempts to search for new antimicrobial agents to combat infections and overcome problems of resistance and side effects of the currently available antimicrobial agents. Action must be taken to reduce this problem such as controlling the use of antibiotics, carrying out research to investigate drugs from natural sources and also drugs that can either inhibit the growth of pathogen or kill them and have no or least toxicity to the host cell are considered conditions for developing new antimicrobial drugs [4, 5].

Antimicrobial peptides (AMP) serves as a potential source for development of novel antimicrobial agents. AMPs are been synthesized by all classes of prokaryotic and eukaryotic microorganisms as a self-defense molecules. The biological interface between fish and their aqueous environment consists of a mucus layer produced from the skin composing of biochemically diverse secretions from epidermal and epithelial cells. The skin secretions creates a protective layer to prevent microbial attacks and other external infections, to have a mechanical protective function, to be involved in osmoregulation and locomotion to play a possible immunological role and to have some function in intra-specific chemical communication [6]. Over the past years, it has also been shown that mucus secreted by the fish skin plays a role in the prevention of colonization by parasites, bacteria and fungi. The antibacterial role of fish skin has been known for many years but previous works on antibacterial tests has been directed towards marine microbial strains. It was reported that epithelial tissues produce antimicrobial peptides (AMPs) which serve as the first line of a host's defense against microbial invasion in a variety of vertebrates including humans[7]. Therefore the present study focuses on isolation of wound pathogens from direct wounds and identification. AMP from skin of *Catla catla* was extracted using two different solvents and its antibacterial activity against wound pathogens were determined.

II. Methodology

Isolation of Wound pathogens

Specimens from wound were collected using sterile swabs and streaked on nutrient agar (Figure-1). Then the colonies were subjected to gram staining and series of biochemical tests like indole, methyl-red, voges-proskauer, citrate utilization and triple sugar ion test. Congo red assay was performed on CRA medium to determine the biofilm producing ability of the isolated pathogens.



Fgure-1: Infected wound from which sample was collected

Collection of fish samples and extraction of Antimicrobial peptides (Poyil et al., 2021)

Live fish of *Catla catla* weighing 470gm was collected from a local fish market Ukkadam, Coimbatore, Tamil Nadu (Figure-2). Skin was removed and cut into small pieces. 20 g of tissue samples were collected and homogenized in 200 ml of solvents (Ethanol and water) separately. The resulting homogenates were filtered using nylon cloth to remove large exoskeleton debris and subjected to centrifugation at 20,000 rpm for 30 min at 4°C (Figure-under reduced pressure until residues emerged.

Figure-2: Collection of *Catla catla*

Antibacterial activity against wound pathogens

Antibacterial activity of the crude extracts of AMP was evaluated by using well diffusion method. Nutrient agar was prepared and sterilized, and poured into plates. Overnight cultures of test pathogens were cultured and 0.1ml of culture solution of each test organisms was streaked throughout the petri plate with the sterile cotton swab by rotating the plate at 60º angle for each streaking. 6mm well borer was used to bore wells on the agar surface of each NA plates. 100µl of AMP was loaded into the well and the plates were incubated in an incubator at 37ºC for 48h. The antibacterial activity was determined in terms of inhibitory zones around the wells in all the plates containing test pathogens. The obtained clear zones were observed and measured in millimetre (mm).

III. Results and Discussion

Isolation and Identification of wound pathogens

After overnight incubation at 37°C, golden yellow colonies and greenish blue colonies were observed on nutrient agar plates. Table-1 shows the tests for identification of wound pathogens and its observations. Based on the colony morphology and biochemical tests the pathogens were found to be *Staphylococcus aureus* and *Pseudomonas aeruginosa.*

Table-1: Identification tests of wound pathogens

S. No	Identification test	Colony-1	Colony-2
1	Colony colour	yellow	greenish blue
2	Gram staining	gram positive	gram negative
3	Indole	-	-
4	Methyl red	+	-
5	Voges proskauer	+	-
6	Citrate utilization	+	+
7	TSI	A/A; no gas formation; H_2S (-)	K/K; no gas formation; H_2S (-)

Biofilm formation assay

Biofilm formation ability of the test pathogen was determined by using congo red assay. Black dry crystalline colonies were observed on both plates, which confirm the organisms are able to produce biofilm. Figure-3 shows the growth of wound pathogen on CRA medium.

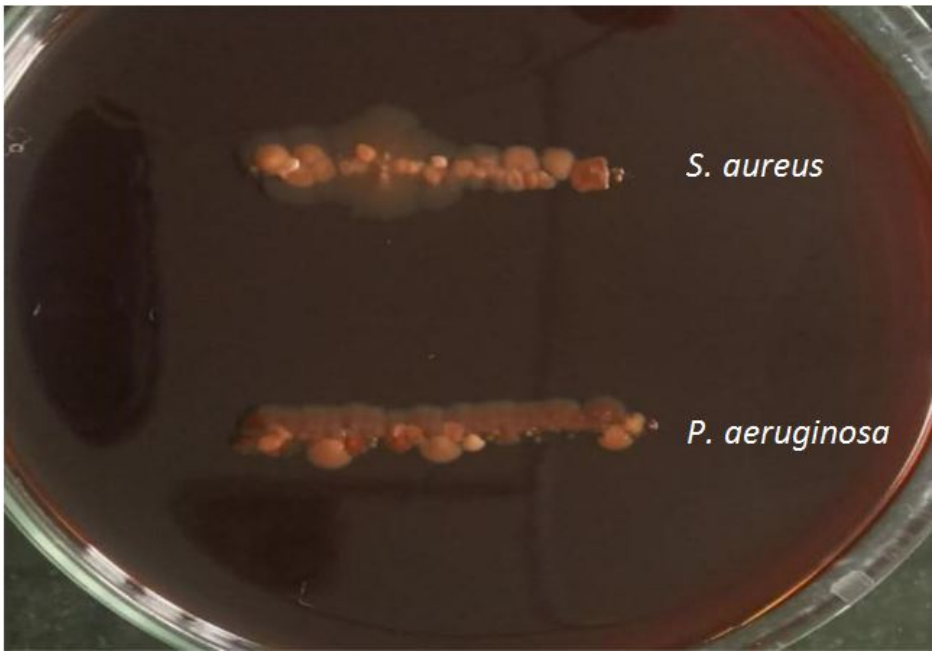


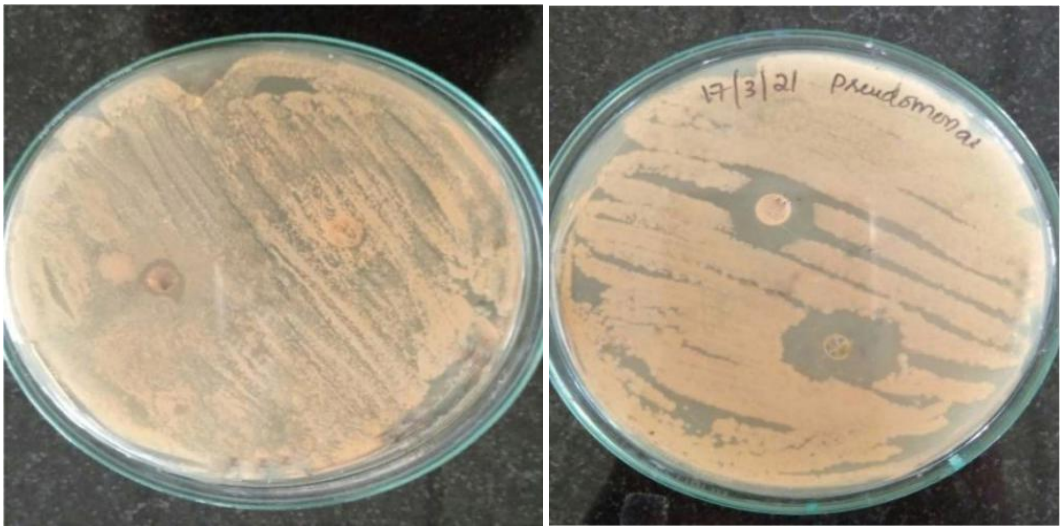
Figure-3: Growth of wound pathogens on CRA media

Antibacterial activity of AMP against wound pathogens

Antibacterial analysis showed ethanolic extract had higher inhibition than aqueous extracts. Aqueous extracts showed 2mm against *S. aureus* and 7 mm against *P. aeruginosa* whereas, ethanolic extracts showed 4mm against *S. aureus* and 11 mm against *P. aeruginosa.*Figure-4 shows

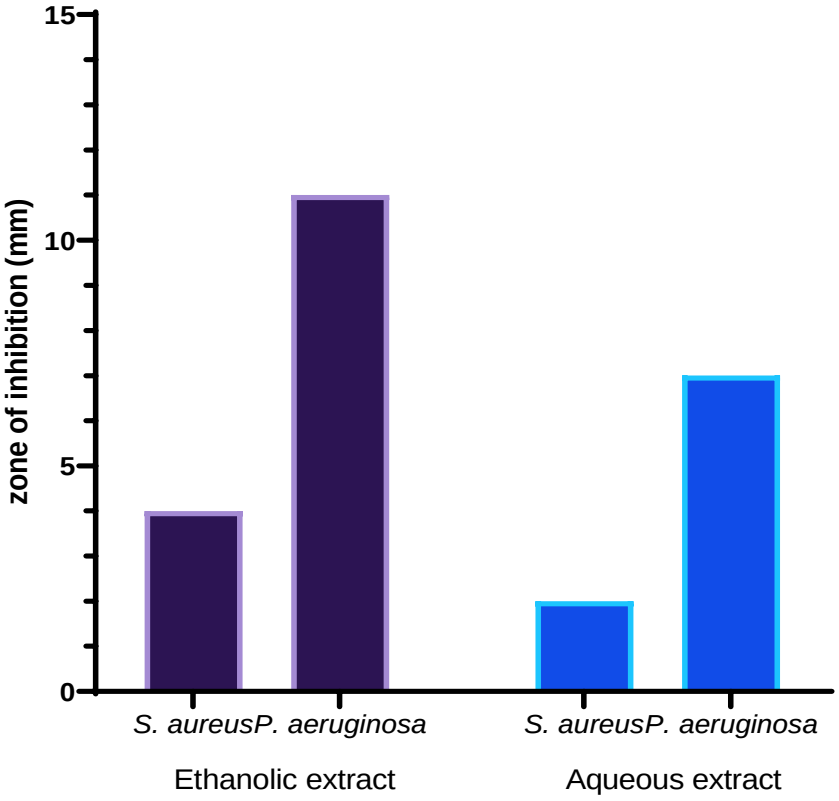
antibacterial activity of AMP extracted using different solvents against wound pathogens and Figure-5 shows the inhibitory zones obtained against wound pathogens.

Figure-2: Antibacterial activity of AMP extracted from *Catla catla*



Staphylococcus aureus *Pseudomonas aeruginosa*

Figure-3: Inhibition of wound pathogens by different solvent extracts of AMP



IV. Discussion

Fishes are living in microbe-rich habitat for which they are bestowed with good defense mechanism in their skin. Fish skin contains antimicrobial peptides as weapons for killing the pathogens. It has been documented that naturally occurring proteins or glycoproteins of non-immunoglobulin nature are present in fish skin and mucus that react with a diverse array of environmental antigens and may confer an undefined extent of natural immunity to fish. Pieces of

evidences suggest that occurrence of infections in fishes are very rare [9]. Thus, it makes many scientists to figure out the defense mechanism in fish skin. Therefore the present study focuses on extraction of AMP from *Catla catla* skin and determining antibacterial activity against wound pathogens.

Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa were found to be the predominant pathogens in wound causing wound infections. Wound infections are found to be of high risk because if left untreated may lead to amputation among diabetic patients. Development of drug resistance among wound pathogens has created necessity for development of new antimicrobials [10]. Antimicrobial peptides are found to be potent source showing promising antimicrobial action. AMP from skin of *Catla catla* was extracted and antibacterial activity was determined against wound pathogens. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the pathogens isolated from wound specimens in the present study.

Owing to its low concentration in specific tissues and the presence of proteases and other non-peptidic molecules, AMP extraction from tissue samples is a difficult step in peptide analysis. Peptide analysis using different methods for isolation, purification, and characterization can be highly time-consuming without sufficient sample preparations. The initial recovery of the peptide allows the sample to be homogenised first. Homogenization utilises the use of organic solvents for peptide recovery. There is no single solvent that can remove a full collection of peptides because of unknown deproteinization abilities and inherent limitations [8]. Since several solvents may have different effects on each tissue types and two different solvents (Ethanol and water) are used in the study. Significant antibacterial activity was observed for AMP extracted using ethanol as solvent.

V. Conclusion

Specimens from wound were collected using sterile swabs and streaked on nutrient agar. Then the colonies were subjected to gram staining and series of biochemical tests for identification. Live fish of *Catla catla* weighing 470gm was collected, homogenized in two different solvents (Ethanol and water). Antibacterial activity of the crude extracts of AMP was evaluated by using well diffusion method. Based on the colony morphology and biochemical tests the pathogens were found to be *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The wound pathogens were also found to be biofilm producer. Antibacterial analysis showed ethanolic extract had higher inhibition than aqueous extracts. Therefore the extracted AMP can be used for development of novel antibacterial drugs for treatment of drug resistant bacterial infections.

VI. References

[1] U. A. Okonkwo and L. A. Dipietro, “Diabetes and wound angiogenesis,” *International Journal of Molecular Sciences*. 2017, doi: 10.3390/ijms18071419.

[2] D. G. Greenhalgh, “Wound healing and diabetes mellitus,” *Clinics in Plastic Surgery*. 2003, doi: 10.1016/S0094-1298(02)00066-4.

[3] S. Sneka, S. P. S, C. Bavya, and D. Latha, “Evaluating the Antibacterial activity of Aqueous , Ethanol and Acetone Extracts of the Chrysanthemum indicum Flowers against Wound Pathogens,” vol. 7, no. 2, pp. 327–333, 2020.

[4] B. Aslam *et al.*, “Antibiotic resistance: a rundown of a global crisis,” *Infection and Drug Resistance*. 2018, doi: 10.2147/IDR.S173867.

[5] S. B. Levy and M. Bonnie, “Antibacterial resistance worldwide: Causes, challenges and responses,” *Nature Medicine*. 2004, doi: 10.1038/nm1145.

[6] J. Schauber and R. L. Gallo, “Antimicrobial peptides and the skin immune defense system,” *Journal of Allergy and Clinical Immunology*. 2008, doi: 10.1016/j.jaci.2008.03.027.

[7] K. Radek and R. Gallo, “Antimicrobial peptides: Natural effectors of the innate immune system,” *Seminars in Immunopathology*. 2007, doi: 10.1007/s00281-007-0064-5.

[8] M. M. Poyil, N. Bari, S. P. S, and M. A. Alfaki, “Extraction of Antimicrobial Peptides (AMPs) from Portunus sanguinolentus Herbst , Perna viridis Linneaus and Octopus indicus Orbigny : Identifying the best Solvent for AMP Recovery and Determining its Anti- ESKAPE Activity,” *J. Res. Med. Dent. Sci.*, vol. 9, no. 1, pp. 20–26, 2021.

[9] O. Hedmon, “FISH MUCUS: A NEGLECTED RESERVOIR FOR ANTIMICROBIAL PEPTIDES,” *Asian J. Pharm. Res. Dev.*, 2018, doi: 10.22270/ajprd.v6i4.389.

[10] R. Edwards and K. G. Harding, “Bacteria and wound healing,” *Current Opinion in Infectious Diseases*. 2004, doi: 10.1097/00001432-200404000-00004.