Antimicrobial Properties of Skin Mucus from Freshwater Fishes

H.D. Kaher

Department of Biosciences, Sardar Patel University, Vallabh Vidhyanagar.

ABSTRACT:
The fishes are living in the medium rich in pathogenic microbes. The mucus secreted by the skin of fish showed more antimicrobial properties. The mucus secreated by the skin of fish showed more antimicrobial properties. The mucus collected from the six freshwater fishes were tested against the four pathogenic bacteria (*E.coli, P.aeruginosa, B.subtilus and S.aureus*) and two pathogenic fungi namely (*Candida albicans* and *Aspergillus niger*) by well diffusion method. The activity was measured in terms of zone of inhibition in mm. The findings of the current investigation showed that the mucus of snake head fish (*Channa punctatus*) may be a potential source antimicrobial agent than the walking catfish (*Clarius batrachus*), grass carp (*Ctenopharyngodon idella*), mrigal (*Cirrhinus mrigala*), bighead carp (*Aristicthys nobilis*), and tilapia (*Oreochromis niloticus*).

Keywords: Mucus, Antimicrobial properties, Pathogenic bacteria, well diffusion method, fish

1. INTRODUCTION

Modern chemotherapeutic techniques have become highly reliable due to the advanced improvements and newer formulations (Ongyeongwei etal.,2013).Extractsand preparation made from the animal origin have been a great healing tool in folk and modern medicine (Kuppulakshmi et al., 2008).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 which combat infections and overcome the problems of resistance with no side effects.The global trade in animal based medicinal products accounts for billions of dollars per year (kunin and Lawton, 1996).

According to WHO, out of 252 traditional medicines, 8.7 % come from animals (Marques, 1997). Indeed, animals are therapeutic arsenals that have been playing significant roles in the healing processes, magic rituals, and religious practices of peoples (Costa and Marques, 2000). All living organisms including fish coexist with a wide range of pathogenic and non- pathogenic microorganisms and therefore, posses complex defense mechanisms which contribute to their survival. One mechanism is the innate immune system that combats pathogens from the moment of their first contact (kimbrell and Beutler, 2001). The specific immunity including antibody and specific cell-mediated responses are significantly less diverse than those of higher (Ellis, 1974; Manning, 1998). 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, which overcome the problems of resistance and side effects. Action must be taken to reduce this problem such as controlling the use of antibiotics, carrying out research to investigate drugs from natural sources. Drugs that can either inhibit the growth of pathogen or kill them and have no or least toxicity to the host cell are considered for developing new antimicrobial drugs. It is well known that the global trade in animal based medicinal products accounts for billions of dollars per year (Kunin and Lawton, 1996).

2. MATERIAL AND METHODS

Fish collection:

Hypophthalmicthys nobilis, cirrhina mrigala, ctenopharyngodon idella and tilapia were obtaind from juhu-krupa farm, pij village, taluka nadiad (dist. Kheda). From there we are transport the fish in polythene bag with airated water and immediately collected the mucus.

Mucus collection:

Mucus collection was carried out by using the method of Ross et al., (2000).the fishes were transfered in to polythene bags conaining 50mM Nacl solution at the rate 10ml for 100g fish. The bags were shaken by hand for 5 minutes to collect mucus and following this treatment fish were retuurned to recover in the beaker. The mucus were immediately transffered into 15ml sterile centrifuge tubes and placed on dry ice.the sample was centrifuged at 1500 rpm for 10 min at 4C and the supernatant obtained was alliqouted into 2ml centrifuge tubes, freeze dried and stored at -20c for further analysis.

Antimicrobial activity by Agar Well Diffusion assay :

In vitro antimicrobial assay was carried out by disc diffusion technique (Bauer et al., 1996). Whatman No.1 filter paper discs with 4 mm diameter were impregnated with known amount (10 µl) of test sample of fish mucus and a standard antibiotic disc. At room temperature (37ºC) the bacterial plates were incubated for 24 h. The fungal plates were incubated at 30ºC for 3 to 5 days for antifungal activity. 0.8% soft agar was seeded with overnight grown culture of 100µl of test organism which was then overlaid on the 2% Luria agar base. With the help of sterile cork borer wells of 6mm diameter were made and filled with 100µL sample under study. Appropriate control was also added in to wells. Plates were incubated at 37 C for 24 h. The diameter of zone of inhibition was observed and measured in millimeter. The spectrum of antimicrobial activity was studied using four different strains of human pathogenic bacteria and two species of fungal pathogens.

3. RESULT

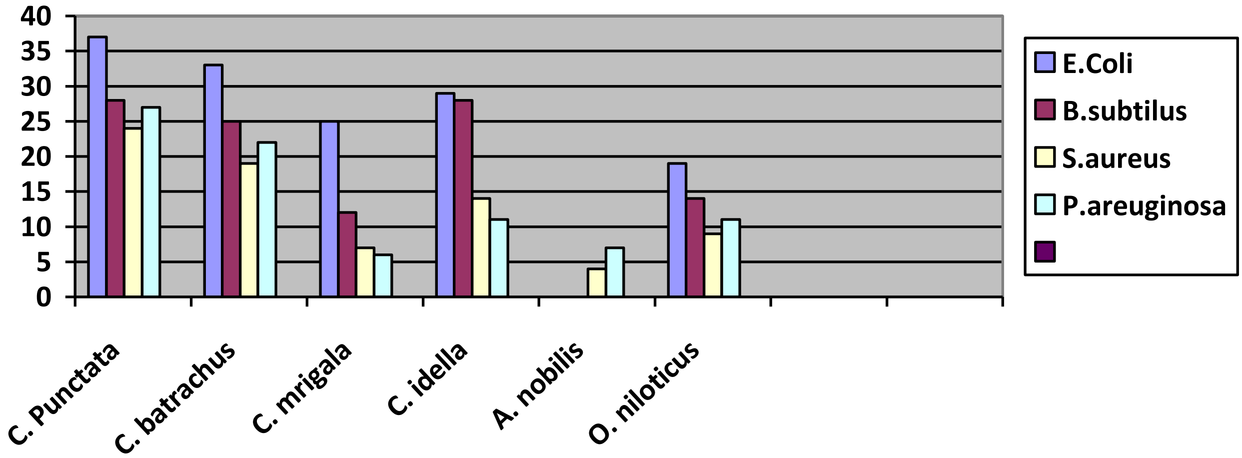
In the present experiment, the antibacterial activity has been analyzed in six diffrent selected fishes against two gram positive bacteria (*B.subtilus, S.aureus*) and two gram negative bacteria bacteria (*E.coli, P.areuginosa*). The activity was measured in terms of zone of inhibition in mm. Mucus secreted by fish play a major role in protection against major infectious agents such as bacteria and fungi haniffa et al., (2014).

The mucus of *Channa Punctata* showed more effect in controlling the growth of gram-negative bacteria *E.Coli* with an inhibition zone of 37 mm in diameter. Next to *Ecoli*, the mucus of *Channa Punctata* showed a better effect on *B.subtilus* having an inhibition zone of 28 mm in diameter. That was followed by the *P.areuginosa* with an inhibition zone of 27 mm in diameter. Among the four bacteria tested *S.aureus* showed very less sensitivity to the mucus of *Channa Punctata* with an inhibition zone of 24 mm in diameter. The mucus of *Clarius batrachus* showed more effect in controlling the growth of gram-negative bacteria *E.Coli* with an inhibition zone of 33 mm in diameter. Next to *Ecoli*, the mucus of *Clarius batrachus* showed a better effect on *B.subtilus* having an inhibition zone of 25 mm in diameter. That was followed by the *P.areuginosa* with an inhibition zone of 22 mm in diameter. Among the four bacteria tested *S.aureus* showed very less sensitivity to the mucus of *Clarius batrachus* with an inhibition zone of 19 mm in diameter.

Table No.1 Antibacterial effect of mucus of selected diffrent fishes analysed by well diffusion assay method. (Zone of inhibition in mm)

Bacteria / *Fish*	*E.Coli*	*B.subtilus*	*S.aureus*	*P.areuginosa*

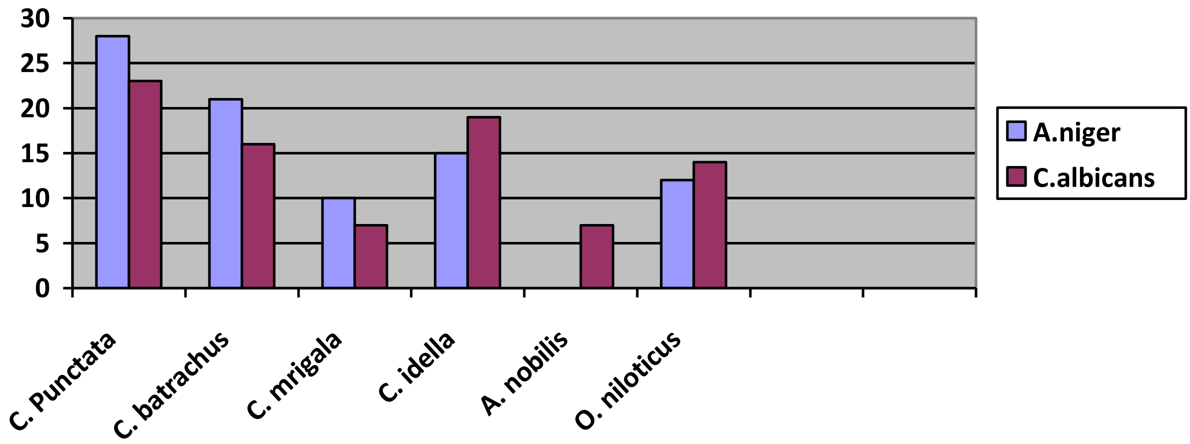
Channa Punctata	**37**	**28**	**24**	**27**
Clarius batrachus	**33**	**25**	**19**	**22**
Cirrhina mrigala	**25**	**12**	**7**	**6**
Ctenopharyngodon idella	**29**	**28**	**14**	**11**
Aristhichthys nobilis	**-**	**-**	**4**	**7**
Oreochromis niloticus	**19**	**14**	**9**	**11**



The mucus of *Cirrhina mrigala* showed more effect in controlling the growth of gram-negative bacteria *E.Coli* with an inhibition zone of 25 mm in diameter. Next to *Ecoli*, the mucus of *Cirrhina mrigala* showed a better effect on *B.subtilus* having an inhibition zone of 12 mm in diameter. That was followed by the *S.aureus* with an inhibition zone of 6 mm in diameter. Among the four bacteria tested *P.areuginosa* showed very less sensitivity to the mucus of *Cirrhina mrigala* with an inhibition zone of 7 mm in diameter.

Table No.2 Antifungalal effect of mucus of selected diffrent fishes analysed by well diffusion assay method. (Zone of inhibition in mm)

Fungi / **Fish**	***A.niger***	***C.albicans***
Channa Punctata	**28**	**23**
Clarius batrachus	**21**	**16**
Cirrhina mrigala	**10**	**7**
Ctenopharyngodon idella	**15**	**19**
Aristhichthys nobilis	**-**	**7**
Oreochromis niloticus	**12**	**14**



The mucus of *Cirrhina mrigala* showed more effect in controlling the growth of gram-negative bacteria *E.Coli* with an inhibition zone of 25 mm in diameter. Next to *Ecoli*, the mucus of *Cirrhina mrigala* showed a better effect on *B.subtilus* having an inhibition zone of 12 mm in diameter. That was followed by the *S.aureus* with an inhibition zone of 6 mm in diameter. Among the four bacteria tested *P.areuginosa* showed very less sensitivity to the mucus of *Cirrhina mrigala* with an inhibition zone of 7 mm in diameter.The mucus of *Ctenopharyngodon idella* showed more effect in controlling the growth of gram-negative bacteria *E.Coli* with an inhibition zone of 29 mm in diameter. Next to *Ecoli*, the mucus of *Ctenopharyngodon idella* showed a better effect on *B.subtilus* having an inhibition zone of 28 mm in diameter. That was followed by the *S.aureus* with an inhibition zone of 14 mm in diameter. Among the four bacteria tested *P.areuginosa* showed very less sensitivity to the mucus of *Ctenopharyngodon idella* with an inhibition zone of 11 mm in diameter.

The mucus of *Aristhichthys nobilis* showed No effect in controlling the growth of gram-negative bacteria *E.Coli* and *B.subtilus*. That was followed by the *P.areuginosa* with an inhibition zone of 4 mm in diameter. Among the four bacteria tested *S.aureus* showed very less sensitivity to the mucus of *Aristhichthys nobilis* with an inhibition zone of 7 mm in diameter.The mucus of *Oreochromis niloticus* showed more effect in controlling the growth of gram-negative bacteria *E.Coli* with an inhibition zone of 19 mm in diameter. Next to *Ecoli*, the mucus of *Oreochromis niloticus* showed a better effect on *B.subtilus* having an inhibition zone of 14 mm in diameter. That was followed by the *P.areuginosa* with an inhibition zone of 11 mm in diameter. Among the four bacteria tested *S.aureus* showed very less sensitivity to the mucus of *Oreochromis niloticus* with an inhibition zone of 9 mm in diameter.

The mucus of *Channa Punctata* showed more effect in controlling the growth of pathogenic fungai *A.niger* with an inhibition zone of 28 mm in diameter. Next to *A.niger*, the mucus of *Channa Punctata* showed a better effect on *C.albicans* having an inhibition zone of 23 mm in diameter. The mucus of *Clarius batrachus* showed more effect in controlling the growth of pathogenic fungai *A.niger* with an inhibition zone of 21 mm in diameter. Next to *A.niger*, the mucus of *Clarius batrachus* showed a better effect on *C.albicans* having an inhibition zone of 16 mm in diameter. The mucus of *Cirrhina mrigala* showed more effect in controlling the growth of pathogenic fungai *A.niger* with an inhibition zone of 10 mm in diameter. Next to *A.niger*, the mucus of *Cirrhina mrigala* showed a better effect on *C.albicans* having an inhibition zone of 07 mm in diameter. The mucus of *Ctenopharyngodon idella* showed more effect in controlling the growth of pathogenic fungai *A.niger* with an inhibition zone of 15 mm in diameter. Next to *A.niger*, the mucus of *Ctenopharyngodon idella* showed a better effect on *C.albicans* having an inhibition zone of 14 mm in diameter. The mucus of *Aristhichthys nobilis* showed No effect in controlling the growth of pathogenic fungai *A.niger* with an inhibition zone of mm in diameter. Next to *A.niger*, the mucus of *Aristhichthys* *nobilis* showed a better effect on *C.albicans* having an inhibition zone of 7 mm in diameter.The mucus of *Oreochromis niloticus* showed more effect in controlling the growth of pathogenic fungai *A.niger* with an inhibition zone of 12 mm in diameter. Next to *A.niger*, the mucus of *Oreochromis niloticus* showed a better effect on *C.albicans* having an inhibition zone of 14 mm in diameter.

4. DISCUSSION

The epithelial surfaces of fish, such as the skin, gills and the alimentary tract provide first contact with potential pathogens. The biological interface between fish and their aqueous environment consists of a mucus layer composing of biochemically-diverse secretions from epidermal and epithelial cells (Ellis, 1999). This layer is thought to act as a lubricant to have a mechanical protective function, to be involved in osmoregulation and play a possible role in immune system of fish. Fish tissue and body fluids contain naturally occurring proteins or glycoproteins of non-immunoglobulin nature that react with a diverse array of environmental antigens and may confer an undefined degree of natural immunity to fish. Antimicrobial peptides are among the earliest developed molecular effectors of innate immunity and are significant in the first line of host defense response of diverse species. Most antimicrobial peptides found through out the animal and plant kingdom are small, functionally specialized peptides (Boman, 1995). Several endogenous peptides with antimicrobial activity from fish, especially from the skin and skin mucus are reported (Park et al., 1997). Endogenous peptides play an important role in fish defense, possess broad spectrum of antimicrobial activity against bacteria, yeast and fungi. The epidermic and the epithelial mucus secretions act as biological barriers between fish and the potential pathogens of their environment (Shephard, 1993). In the present study, variation in their antimicrobial activity was observed among the fish mucus. This may be due to the variation in the relative levels of lysozyme, alkaline phosphatase, cathepsin B and proteases of the epidermal mucus of all fish species (Subramanian et al., 2007).

5. CONCLUSION

Falling in line with the above observation, the indigenous fish species such as *Channa Punctata* and *Clarius batrachus* show higher antimicrobial activity than that of the exotic fish species such as *C. idella*. This is the first report on the antimicrobial activity of skin mucus of cultivable indigenous fishes of India. Moreover the mucus of fish possesses antimicrobial agents which could be used to formulate new drugs for the therapy of infectious diseases caused by pathogenic and opportunistic microorganisms. These properties of mucus suggest that it may be beneficial in aquaculture and human health-related applications. Further studies are needed to isolate the bioactive compounds (antimicrobial substances) from the mucus of these cultivable fish species and the mechanism of antimicrobial action.

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