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Antibacterial properties of fish mucus from *Channa punctatus* and *Cirrhinus mrigala*

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Abstract. – Extracts and preparations made from the animal origin were used extensively in folk and modern medicine for treating many human diseases. In the present study efforts have been made to find the antimicrobial effect of the mucus of two bottom dwelling fresh water fishes namely, *Channa punctatus* and *Cirrhinus mrigala*. Fish mucus were tested against ten pathogenic bacteria such as *Escherichia coli*, *Klebsiella oxytoca*, *Klebsiella pneumoniae*, *Lactobacillus vulgaris*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Salmonella paratyphi*, *Salmonella typhi*, *Staphylococcus aureus* and *Vibrio cholera*. The activity was measured in terms of zone of inhibition in mm. The mucus collected from *Cirrhinus mrigala* shows a strong inhibiting activity than the mucus of *Channa punctatus*.

Key Words:

Antibacterial activity, Fish mucus, *Channa punctatus, Cirrhinus mrigala.*

Introduction

Chemicals from nature have been a part of human civilization ever since our early ancestors began exploiting natural compounds to improve and enrich their own lives[1]. Inspite of modern improvements in chemotherapeutic techniques, infectious diseases are still an increasingly important public health issue[2]. 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. The healing of human ailments by using therapeutics that is obtained from animals or ultimately is derived from them. It is well known that the global trade in animal based medicinal products accounts for billions of dollars per year[3]. 252 traditional medicines have been selected by WHO, of which 11.1% come from plants and 8.7% from animals[4].

The biological interface between fish and their aqueous environment consists of a mucus layer composing of biochemically diverse secretions from epidermal and epithelial cells[5,6]. This layer is throught to act as a lubricant[7], to have a mechanical protective function[8], to be involved in osmoregulation and locomotion to play a possible immunological role[9] and to have some function in intra-specific chemical communication[10]. Over the past years, it has also been shown that mucus plays a role in the prevention of colonization by parasites, bacteria and fungi[11-13]. The antibacterial role of fish mucus 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 molecules which serve as the first line of a host's defense against microbial invasion in a variety of vertebrates including humans[14].

As a result of indiscriminate use of antimicrobial drugs in the treatment of infectious diseases, microorganisms have developed resistance to many antibiotics. There is a need to develop alternative antimicrobial drugs. One approach is to screen local medicinal animals which represent a rich source of novel antimicrobial agents. Antibacterial activity in mucus has been demonstrated in several fish species[15]. Yet this activity

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seems to vary from species to species and can be specific towards certain bacteria[16]. Though studies are available on the antimicrobial activity of fishes but there were no investigations regarding the bottom dwelling fishes. In the present study effort was made to find the antibacterial activity of the two bottom dwelling fishes *Channa punctatus* and *Cirrhinus mrigala*.

Materials and Methods

Growing live fishes approximately 6 months old, weigh about 500 gms each *Channa punctatus* and *Cirrhinus mrigala* were purchased from the nearby culture pond. The purchased fishes were acclimatized to laboratory condition in well water and they were maintained for 4 days. After 4 days these fishes were used for mucus collection.

Collection of mucus from fish. Mucus was carefully scraped from the dorsal body using a sterile spatula. Mucus was not collected in the ventral side to avoid intestinal and sperm contamination. The collected fish mucus was stored at 4°C for further use. The mucus samples were collected aseptically from the fish and thoroughly mixed with equal quantity of sterilized physiological saline (0.85% Nacl) for the antimicrobial studies.

In vitro antimicrobial evaluation. *In vitro* antimicrobial evaluation of fish mucus of *C. punctatus* and *C. mrigala* were carried out against ten bacterial strains-*Escherichia coli, Klebsiella oxytoca, Klebsiella pneumoniae, Lactobacillus vulgaris, Proteus mirabilis, Pseudomonas aeruginosa, Salmonella paratyphi, Salmonella typhi, Staphylococcus aureus* and *Vibrio cholera*. All bacterial strains were obtained from the Department of Medical Microbiology, Rajah Muthiah Medical College, Annamalai University, India.

Determination of antimicrobial assay. Antimicrobial activity was measured using the standard method of diffusion disc plates on agar. 0.1 ml of each culture of bacteria was spread on agar plate surfaces. For antibacterial assays, all bacterial strains were grown in Muller Hinton Broth medium for 24 hrs at 37°C. The concentration of bacterial suspensions was adjusted to 10^8 colony forming units (10^8 cfu/ml) in Muller Hinton Agar. Paper discs (6 mm in diameter) were impregnated on the agar to load 10 µl of each sample. The impregnated disks were placed on the medium suitably spaced apart and the plates were incubated at 5°C for 1 hr to permit good diffusion and then transferred to an incubator at 37°C for 24 hrs. The results were recorded by measuring the zones of growth inhibition surrounding the disc. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. All data on antimicrobial activity are the average of triplicate analyses. In order to determine the antibacterial effect of the fish mucus, chloramphenicol (10 µg/ml/disc) were measured after incubation for 24 hrs at 37°C.

Results

To elucidate the antibacterial activity of the fish mucus isolated from the two bottom dwelling fresh water fishes *Channa punctatus* and *Cirrhinus mrigala*, we used a classical inhibition assay on thin agar. The results of the antibacterial activity of mucus of the *Channa punctatus* and *Cirrhinus mrigala* are presented in the Table I. The mucus collected from both bottom dwelling fresh water fishes shows a strong inhibition in the growth of tested bacteria. Maximum zone of inhibition was observed against *Vibrio cholera* (30 mm, 28 mm in diameter), followed by *Staphylococcus aureus* with a inhibition zone of 25, 23mm respectively. On the contrary least inhibition was observed against *Salmonella paratyphi* (7 mm, 9 mm), whereas the other bacteria show a significant inhibition in their growth in the order of *Salmonella typhi* (19 mm, 24 mm), *Lactobacillus vulgaris* (19 mm, 20 mm), *Pseudomonas aeruginosa* (20 mm, 14 mm), *Proteus mirabilis* (20 mm, 12 mm), *Klebsiella oxytoca* (12 mm, 32 mm), *Escherichia coli* (11 mm, 18 mm) and *Klebsiella pneumoniae* (13 mm, 11 mm) respectively.

The comparative antibacterial effect of the mucus of the two bottom dwelling fishes *Channa punctatus* and *Cirrhinus mrigala* with standard drug Chloramphenicol are shown in the Figure 1.

Discussion

Synthetic drugs and food preservatives are notably affected the ecosystem to a considerable extent, due to their higher persistency and constant accumulation in the biological system. To

Table I. Antibacterial activity of the mucus of *Channa punctatus* and *Cirrhinus mrigala*.

Microorganisms	*Channa punctatus* 100 µl/disc	*Cirrhinus mrigala* 100 µl/disc	*Chloramphenicol* 10 µg/ml/disc
E. coli	11	18	20
K. oxytoca	12	32	18
K. pneumoniae	13	11	21
L. vulgaris	19	20	18
P. mirabilis	20	12	22
P. aeruginosa	20	14	20
S. paratyphi	7	9	18
S. typhi	19	24	21
S. aureus	25	23	20
V. cholera	30	28	22

All the values were the mean of three experiments. The values given are the diameter of zone of inhibition (mm) including disk diameter of 6 mm.

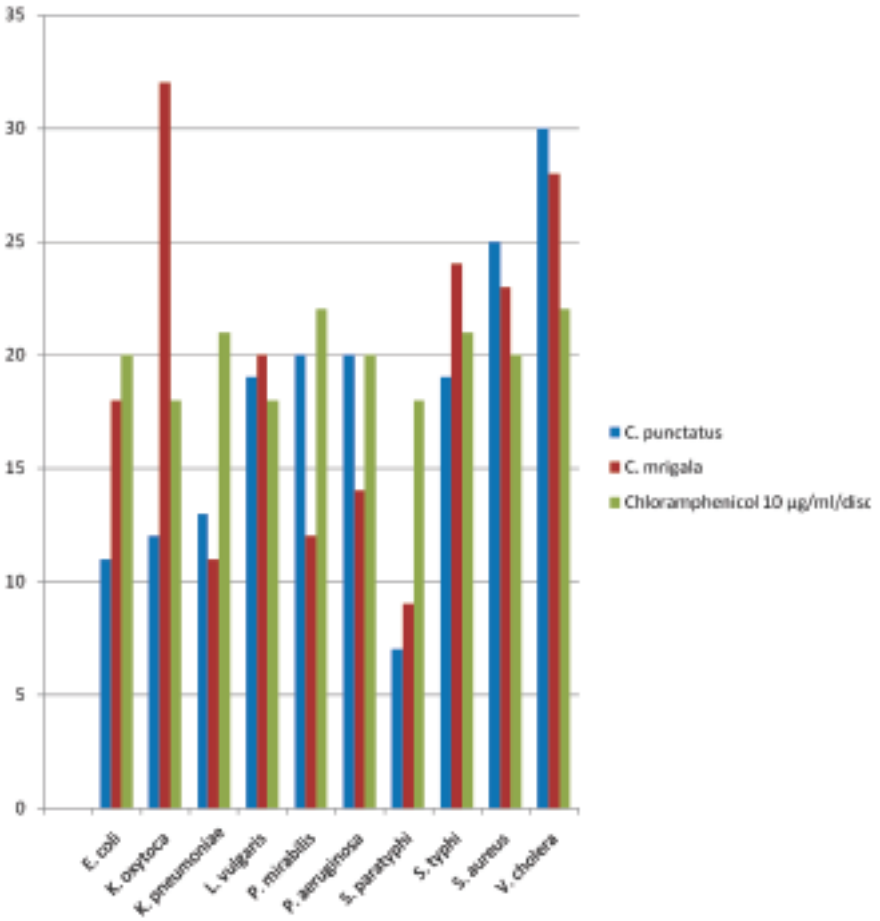

Figure 1. Graph showing the comparative effect of the mucus of two fishes with a standard drug chloramphenicol.

overcome this, considerable investigations are being carried out to develop safer source. Animals have been used as medicinal resources for the treatment and relief of a myriad of illnesses and diseases in practically every human culture[17]. Innate antimicrobial compounds with a broad antimicrobial effect have been identified in a variety of multicellular organisms[18], ranging from insects[19] to several groups of vertebrates e.g. fishes[20], amphibians[21] and mammals[22].

Amphibian skin has probably been the most exploited for their antimicrobial compounds. Amphibian granular glands produce certain secretions that might be effective against microbial and fungal infections[23]. Antimicrobial compounds have been found associated with and dispersed from the epithelial mucus-secreting cells of fishes[20].

Fish by-products are rich in potentially valuable proteins, minerals, enzymes, pigments or flavours[24]. Some antimicrobial agents are present in the mucus of bony fishes which bind to the microbes and destroy it[18,25-27]. Hellio et al[24] reported that lysozyme isolated from fish was an enzyme with bacteriostatic properties and was upiquitous in its distribution among living organisms.

Sloughing of microbes in the mucus contains many antibacterial substances including antibacterial peptides, lysozyme, lectins and proteases[28]. According to Boman[29] and Andreu and Rivas[30] most of the antimicrobial peptides kill bacteria by a common mechanism, which involves direct electrostatic interactions with negatively charged phospholipids on microbial cell membranes followed by physical disruption and solubilization.

In the present study the mucus isolated from *Channa punctatus* and *Cirrhinus mrigala* shows a strong inhibiting effect on the tested microorganisms. This may be due to the pore forming properties against several bacterial strains and these suggested that fish secrete antibacterial proteins able to permeablize the membrane of the target cell and thus act as a defense barrier. The antibacterial activity may be due to the antibacterial glycoproteins present in the mucus able to kill bacteria by forming large pores in the target membrane[27]. Fish mucus is believed to play an important role in the prevention of colonization by parasites, bacteria and fungi and thus acts as a chemical defense barrier.

The results of the present study support the folkloric usage of fishes and suggest that the mucus of fishes possess certain constituents with antimicrobial agents in new drugs for the therapy of infectious diseases caused by pathogens. The mucus secreted from fishes serves as a wall between the internal and external environment which is implicated as a lubricant having mechanical protective function. The mucus collected from *Channa striatus* and *Cirrhinus mrigala* which shows broad spectrum of antibacterial activity can be subjected to further evaluation to analyses the chemical composition as well as to reveal the mode of action on bacteria.

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