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Antibacterial activity of fish mucus from *Clarias batrachus* (Linn.) Against selected microbes

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ABSTRACT

Fish one of the diverse group of animals, that are highly specialized for their aquatic existence. Being fish are in aquatic intimate contact with such an environment that contains very high concentrations of bacteria and viruses. The immune system is composed of numerous organs and cells that act together in a dynamic network in the defense against infection, disease and foreign substances. Fish mucus were tested by using disc diffusion technique against seven pathogenic bacteria such as *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aerugenosa, Proteus vulgaris, Salmonella paratyphi A, Salmonella paratyphi B, Bacillus subtilis*. The activity was measured in terms of zone of inhibition in mm.The mucus from *C.batrachus* showed broad spectrum of antibacterial activity.

KEY WORDS: Antibacterial activity, Fish mucus, *Clarias batrachus.*

INTRODUCTION

Inspite of modern improvements in chemotherapeutic techniques, infectious diseases are still an increasingly important public health issue (World Resources Institute, 2000). 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 mucus protects the skin from pathogens and suspended particles and its alarm substance mucin has potential of antimicrobial and noxious properties (Knouft et al., 2003). Mucus plays an important role in the prevention of colonization of parasites, bacteria and fungi (Yan et al., 2000; Estari Mamidala, 2013). The functional property of the mucus depends on its capacity to form gel on the epithelial surface (Bragadeeswam et al., 2011). This mucus is secreted by the epidermal goblet cells, composed mainly of water and gel forming macromolecules such as mucins and other glycoproteins (Martinez et al., 2006) In addition fish mucus also contains a variety of biologically active substances such as lysozyme, lectins, flavoenzymes, immunoglobulin. 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 (Villarroel et al., 2007 and Ashwin et al, 2015).

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The present investigation was carried out to determine antimicrobial activity of fish *C. batrachus* mucus against some microbes.

MATERIAL AND METHODS

Fish collection and Maintenance

C.batrachus collected by the fishermen from river Krishna were used for the present investigation. The fish were stocked into the 16 x 8 x 10 capacity glass aquaria and acclimatized to laboratory conditions in a tap water. They were maintained for one week. During this period the fish were fed with commercial feed once a day at ad libitum. Every day 50% of the water was changed. After one week of acclimatization the fish were used for mucus collection. Only healthy fish were used for mucus collection, dead fish or fish with skin lesions were removed from the tanks.

Mucus Collection

Fish was starved for one day prior to mucus collection. Before collection of mucus fish were kept out of water in specimen tray for 1 hour. After one hour mucus secreted on the epidermal surface of the body of fish was collected as sample. Mucus was carefully scraped from the dorsal body surface using a sterile spatula. Mucus was not collected in the ventral side to avoid intestinal contamination. The collected fish mucus was stored at 4^0 c for further use.

Antimicrobial Evaluation

Invitro antimicrobial evalution of fish mucus of *C.batrachus* were carried out against seven bacterial strains. *Klebsiella pneumoniae, Staphylococcus aureus, Pseudomonas aerugenosa, Proteus vulgaris, Salmonella paratyphi A, Salmonella paratyphi B, Bacillus subtilis.* All bacterial strains were obtained from the Department of microbiology, Krishna Institute of Medical Science, Karad.

Antimicrobial Assays

The spectrum of antimicrobial activity was studied using the above mentioned bacteria which are designated as human pathogens. One antibiotic, Penicillin used against pathogenic bacteria as control In vitro antibacterial assays was carried out by the disc diffusion technique(Irobi et al,1996)) Whatman No. 1 filter paper disc with 4 mm diameter were used. 0.1 ml of each culture of bacteria was poured on agar plate surfaces. For antibacterial assays, all bacterial strains were grown in nutrient agar medium for 24 hrs at 37 $\pm 0.1^0$c. The concentration of bacterial suspensions was adjusted to 10^8 colony forming units (10^8 cfu/ml) in Muller Hinton agar. Paper disc (4 mm in diameter) were impregnated on the agar to load 10 µl of each sample. The impregnated disc were placed on the medium suitably spaced apart and the plates were incubated at 5^0c for 20 minutes to permit good diffusion and then transferred to an incubator at 37 ± 0.1^0c for 24 hrs. The results were recorded by measuring the zones of growth inhibition surrounding the disc. All data on antimicrobial activity are the average of triplicate analyses. In order to determine the antimicrobial effect of the fish mucus and penicillin (10µg/ml/disc) were measured after incubation for 24 hrs at 37 $\pm 0.1^0$c.

RESULTS

The results of the antibacterial activity of mucus of *C. batrachus* and penicillin (10µg/ml/disc) are presented in the Table I. The mucus collected from fish showed a strong inhibition in the growth of tested bacteria. Clear inhibition zones around the discs indicated the presence of antimicrobial activity. Maximum zone of inhibition was observed against *Klebsiella pneumoniae* (38 mm in diameter), followed by *Staphylococcus aureus* with inhibition zone of 20 mm respectively. The comparative antibacterial effect of the mucus of *C.batrachus* with standard drug penicillin shown in Plate 1. The mucus sample collected from *C.batrachus* showed a significant activity with regard to gram positive as well as gram negative bacteria. The zone of inhibition values of *C.batrachus* were much more than the positive control (Penicillin) tested and the inhibiting effect of mucus of *C.batrachus* against seven bacterial strains are given in Table 1 and the graphical representation is shown in Plate 1.

Table-1. Antibacterial activity of the mucus of fish *Clarias batrachus*

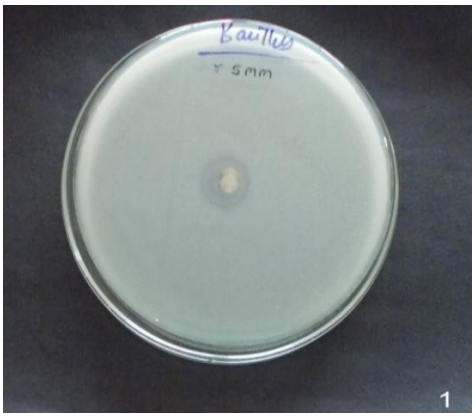
S.No.	Bacterial Pathogen	SD
1.	Penicillin (Control)	0.124 ±0.3
2.	*Klebsiella pneumoniae*	2.160 ±36
3.	*Staphylococcus aureus*	1.699 ±20.6
4.	*Pseudomonas aerugenosa,*	0.816 ±5
5.	*Salmonella paratyphi B*	0.205 ±0.53
6.	*Salmonella paratyphi A*	0.163 ±0.3
7.	*Proteus vulgaris*	0.124 ±0.73
8.	*Bacillus subtilis*	0.124 ±0.36

Values are mean of three replicates ± S.D.

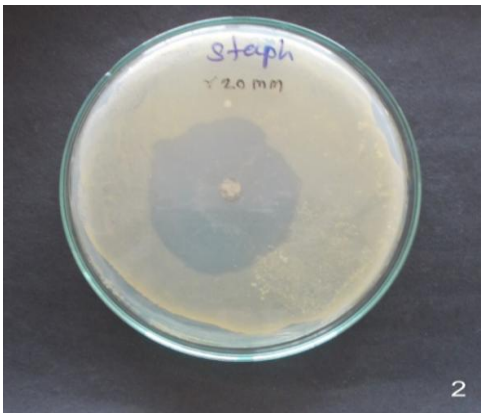
DISCUSSION

Usage of natural chemicals is an ancient practice in human civilization. Exploration of natural compounds from different sources is a continuous task to improve and enrich their own lives (Agosta, 1996).Extracts and preparation made from the animal origin have been a great healing tool in folk and modern medicine (Kuppulakshmi et al., 2005). The development of resistance by a pathogen to many of the commonly used antibiotics provide an impetus for further attempts to search for new antimicrobial

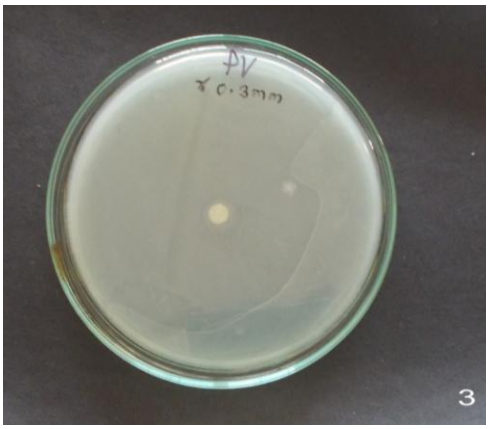
Plate-1: The comparative antibacterial effect of the mucus of *C.batrachus* with standard drug penicillin



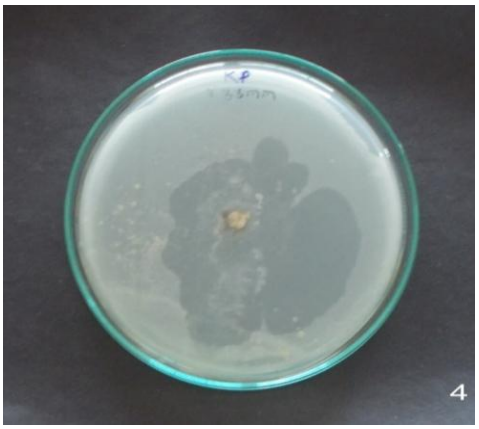
Bacillus subtilis



Staphylococcus aureus



Proteus vulgaris



Klebsiella pneumoniae

agents which combat infections and overcome the problems of resistance with no side effects.

Many workers previously demonstrated the antimicrobial property of epidermal mucus in fishes. e.g. in *Cyprinus myxine glutine*_by subramanain_et al., (2009). In cat fish –*Arius maculates* by Manivasagan et al., (2009), in eel fish- *Anguilla anguilla* by Bragadeeswaran and Thangraj (2011). In the present investigation the attempt was made to evaluate the antibacterial activity of fish mucus from *C.batrachus.*

Biochemical nature of the mucus of fish have been investigated by many researchers. According to Pickering (1977) and Ellis (1999) mucus layer is a biological interface between fish and their aqueous environment that consist of biochemical diverse secretions from epidermal and epithelial cells. Videler et al., (1999) stated that mucus secreted by external epithelial goblet cells posses biochemical antibiotic compounds. Ellis (2001) reported variety of antimicrobial compounds such as AMPs, lysozymes, proteases and lectins in mucus secreted by epidermal epithelial cells. The mucus secreted by epidermal goblet cells composed mainly of water and gel forming macromolecules such as mucins and other glycoproteins, Martinez et al., (2006). In the present investigation through the biochemical analysis of mucus from C. batrachus was not done, it is agreed that it may consists the same biochemical antibiotic compounds reported earlier by number of investigators.

Several functions for this sticky layer have been suggested earlier by number of investigators. According to Rosen and Cornford (1971) this mucus layer acts as a lubricant. Cameron and Endean (1971) confirm its mechanical protective role. According to Fleteher (1978) mucus involved in osmoregulation and locomotion to playing a possible immunological role. Mucus protects the skin from pathogens and suspended particles and its alarm substance mucin has potential of antimicrobial and noxious properties (Knouff et al., 2003) Yan et al., (2010) demonstrated the role of mucus in the prevention of colonization of particles bacteria and fungi. Bragadeeswaran and Thangaraj (2011) stated that the functional properties of the mucus depend on its capacity to form a gel on the epithelial surface. The results obtained in the present investigation

clearly indicated that mucus collected from fish *C. batrachus* may contain some active ingredients definite antibacterial activity. However, which showed broad spectrum of antibacterial activity against all test bacteria which can be subjected to further evaluation to analyse the chemical composition as well as to reveal the mode of action on bacteria.

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Conflict of Interests:

The authors declare that there is no conflict of interests regarding the publication of this paper.

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