CHEMICAL CONSTITUENTS AND ANTIBACTERIAL ACTIVITY OF ZEBRAFISH MUCUS AGAINST DISEASE CAUSING PATHOGENS

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Abstract : . To study the antibacterial action of zebrafish and their chemical constituent of Zebrafish (*Danio rerio*) mucus. Fish mucus plays a very vital role in the hindrance of colonization by parasites, bacteria and fungi. Epidermal mucus was obtain from Zebrafish, centrifuged and the chemical composition of epidermal mucus was analysed by FTIR study. The invitro antibacterial activity in opposition to human pathogens *Staphylococcus aureus*, *Escherichia.coli*, *Bacillus subtilus*. Totally three human pathogens have tested in opposition to fish mucus, out of that *Staphylococcus aureus* (4.3 cm) have prove to be the more sensitive to the mucus follow by *Escherichia.coli* (4.1cm) and *Bacillus subtilus* (0.1cm) correspondingly. The current investigation has exposed that positive growth in the fish mucus extracts against human being pathogens. A further attempt is in progress for the purification and separation of bioactive compound in order to create the chemo therapeutic applications.

IndexTerms **– Antibacterial, invitro, colonization, zebrafish.**

I. INTRODUCTION

II. Fish have specific humeral and cellular immunity to resist against bacterial diseases. The products obtained from fish are rich in proteins, minerals, enzymes, pigments and flavors. The skin and the mucus of the fish are used for research on biologically active compounds is an interesting factor(Fletcher et al 1978).The mucus layer covers the surface of the external body to reduce body friction against water and to prevent from abrasion injury(Ellis et al 1999). It has a mixture of biologically active substances in the mucus and considerably act as a humeral defense factor, since the fish immunity is less difficult than that of higher animals(Bragadeeswaran et al 2011) . In the past years, fish mucus plays an role in the avoidance of colonization by parasites, bacteria and fungi (Rosen et al 1971). Chemicals obtain from nature is been part of human culture, since natural relations began exploiting natural compounds to get better and enrich their own lives. Even though there are many modern types of equipment, infectious diseases are still increasing important public health issues (Manivanan et al 2011). Recently the progress of the resistance by a pathogen to many of the commonly used antibiotics gives way for further attempts to search for new antimicrobial agents to resist infections and overcome problems of resistance and side effects with the currently available antimicrobial agents. The curative of the human ailments by using therapeutic that it is obtained from animals is need of the present days (Noya et al 1995). The mucus layer of the fish act as a lubricant and mechanical protective agent and also involved in osmo-regulation and immunological property (Fletcher et al 1978). It was reported that epithelial tissues generate antimicrobial molecules which serves as the first line of a host's defense against microbial invasion in a variety of vertebrates including humans (Ebran et al 2000). As a result of random use of antimicrobial drugs in the treatment of infectious diseases, there is a need to increase different antimicrobial drugs (Alexander et al 1992). The antibacterial activity of the fish mucus has been reported in many fish species. Still this activity seems to differ from species to species and can be specific towards certain bacterial species (Austin et al 1988). In the present study effort has been made to find the chemical constituents and antibacterial activity of the Zebra fish (*Danio rerio*).

II.MATERIALS AND METHODS

2.1. Collection of mucus from fish

The fish *Danio rerio* was purchased from an aquarium in Coimbatore. The purchased fishes were acclimatize in laboratory situation using tap water and they were maintained for one week. After four days these fishes were used for mucus collections. Mucus was carefully scraped from the dorsal body using a sterile spatula. Mucus was not collected on the ventral side to avoid intestinal and sperm contamination. The mucus samples were collected aseptically from the fish and thoroughly mixed with equal quantity of sterilized physiological saline for the antibacterial studies (Subramaniam Bragadeeswaran et al, 2011).

2.2. INVITRO ANTIMICROBIAL EVALUATION:

Invitro antimicrobial evaluation of fish mucus of *Danio rerio* were carried out against three different bacterial strains *Staphylococcus aureus*, *Escherichia. Coli* and *Bacillus subtilus*. All the bacterial strains were obtained from the Microbial laboratory, Coimbatore.

2.3. DETERMINATION OF ANTIMICROBIAL ASSAY:

Antimicrobial activity was measured using well-diffusion and disc diffusion methods respectively. The results were recorded by measuring zones of growth inhibition surrounding the well. Clear inhibition zones around the well indicated that the presence of antimicrobial activity. In order to determine the antibacterial effect of Ampicillin were measured after incubation for 24 hours at 37^0c.

2.4. FOURIER TRANSFORM-INFRARED SPECTRAL ANALYSIS:

FT-IR Spectroscopy of zebra fish (*Danio rerio*) mucus sample was mixed with dried potassium bromide (KBr) and compressed further to prepare as salt disc for reading the spectrum.

III. RESULTS AND DISCUSSION

3.1ANTIBACTERIAL ACTIVITY:

To evaluate the antibacterial activity of the fish mucus of *Danio rerio*, we used a classical inhibition assay on thin agar. The results of the antibacterial activity in mucus of the *Danio rerio* are presented in Table –1. The mucus collected from the fish *Danio rerio* shows a strong inhibition in the growth of the tested bacterium. Maximum zone of inhibition was observed against *Staphylococcus aureus* with (4.3 cm) followed by *Escherichia. Coli* (4.1 cm) respectively. On the contrary we didn't get any significant results against *Bacillus subtilus*. The picture depicting the zone of inhibition against the tested bacteria are shown in Fig-1

3.2 FTIR Analysis:

The FT-IR spectrum of the fish mucus sample shows the presence of amine group-aromatic compound,aliphatic alkyl group and polysaccharides (carbohydrates). The IR spectra are attributed to the alkyl amini with polysaccharides in the mucus of the fish.

Most organisms contain antimicrobial property, yet most of the antimicrobial agent that have been secluded from the aquatic source have not been lively enough to race with the usual antimicrobials obtained from microorganisms (Lemaitre et al 1996). Artificial drugs and food preservatives have particularly affected the ecosystem to a large extent, due to their higher persistency and continuous accumulation in the biological system. To overcome these problems, substantial investigations are being carried out to increase safer sources. Fish mucus is a multifunctional substance which plays a key role in communication struggle to disease, respiration, ionic and osmotic parameters, feed, nest building, reproduction and excretion etc. (Cameron et al 1973). In the current study the mucus of the *Danio rerio* shows higher antibacterial activity against human pathogens. Fish by products are rich in potentially important components (Noya et al 1995) and inhibition effect may be due to the pore forming property against several bacterial strains. These studies suggested that fish secrete antibacterial proteins and are capable to permeable the membrane of the objective cell and thus act as a defense obstacle. The antibacterial activity of zebra fish may be due to the antibacterial glycoprotein present in the mucus able to kill the bacteria by forming large pores in the target cell membrane (Rosen et al 1971). So the results of the present study reveals that the mucus of the fish have certain constituent with the antimicrobial agent in the new drugs progress for the therapy of infectious diseases caused by pathogens (Cole et al 1997). Evidently report of Hellio et al in the year (2002) suggested that the lysozyme secluded from the fish was an enzyme with bacteriostatic property and was everywhere in its distribution among living organisms (Hellio et al 2002). The mucus secreted from the fishes serve as a wall between the internal and the external environment which is concerned as a lubricant having mechanical protective function (Kuppulakshmi 2008). The mucus composed from zebra fish (*Danio rerio*) shows the wide spectrum of chemical composition and antibacterial activity and be able to be subjected to further estimate, examine and reveal the mode of action on bacteria.

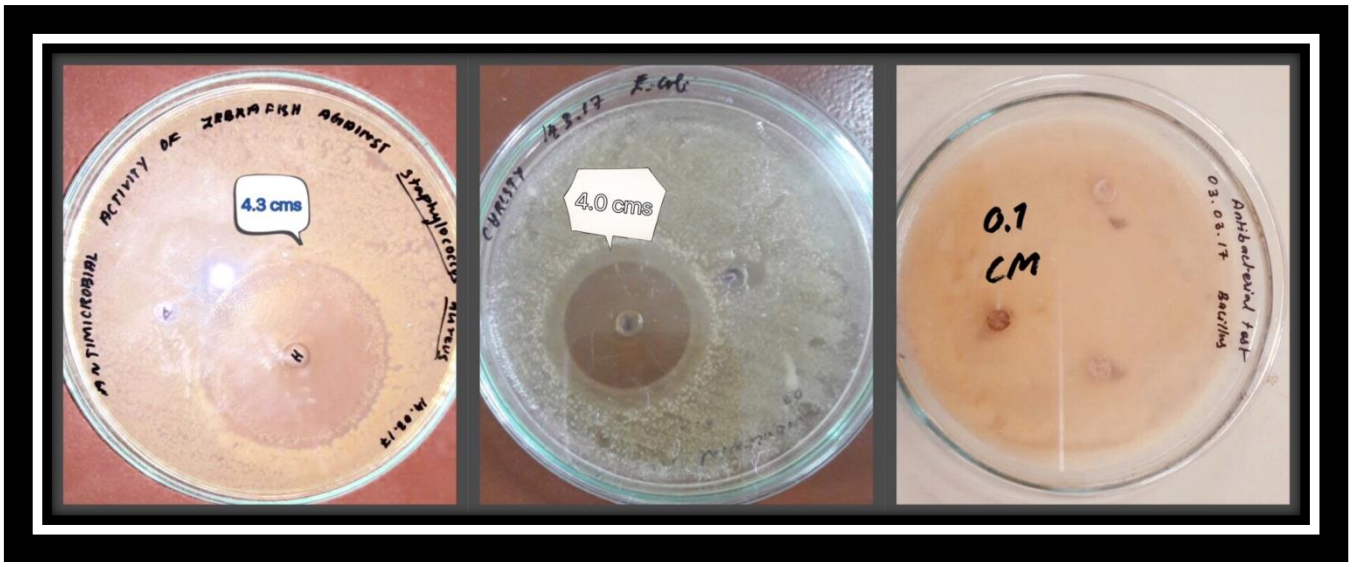
Table 1

Table 1. Represent the zone of inhibition range in cm using different solvents (Hexane, Acetone, Methanol) against three human pathogens.

S.No	Name of the Organism	Hexane	Acetone	Methanol
1	Staphylococcus aureus	4.3	0	0
2	Escherichia. Coli	4.1	0	0
3	Bacillus subtilus	0.1	0	0

Fig 1. Showing the Zone of inhibition (cm) in Zebra fish Mucus sample using different solvents:

A **B** **C**



A. Picture showing the Zone of Inhibition in Zebra fish against Staphylococcus aureus.

B. Picture showing the Zone of Inhibition in Zebra fish against E.coli.

C. Picture showing the Zone of Inhibition in Zebra fish against Bacillus subtilus.

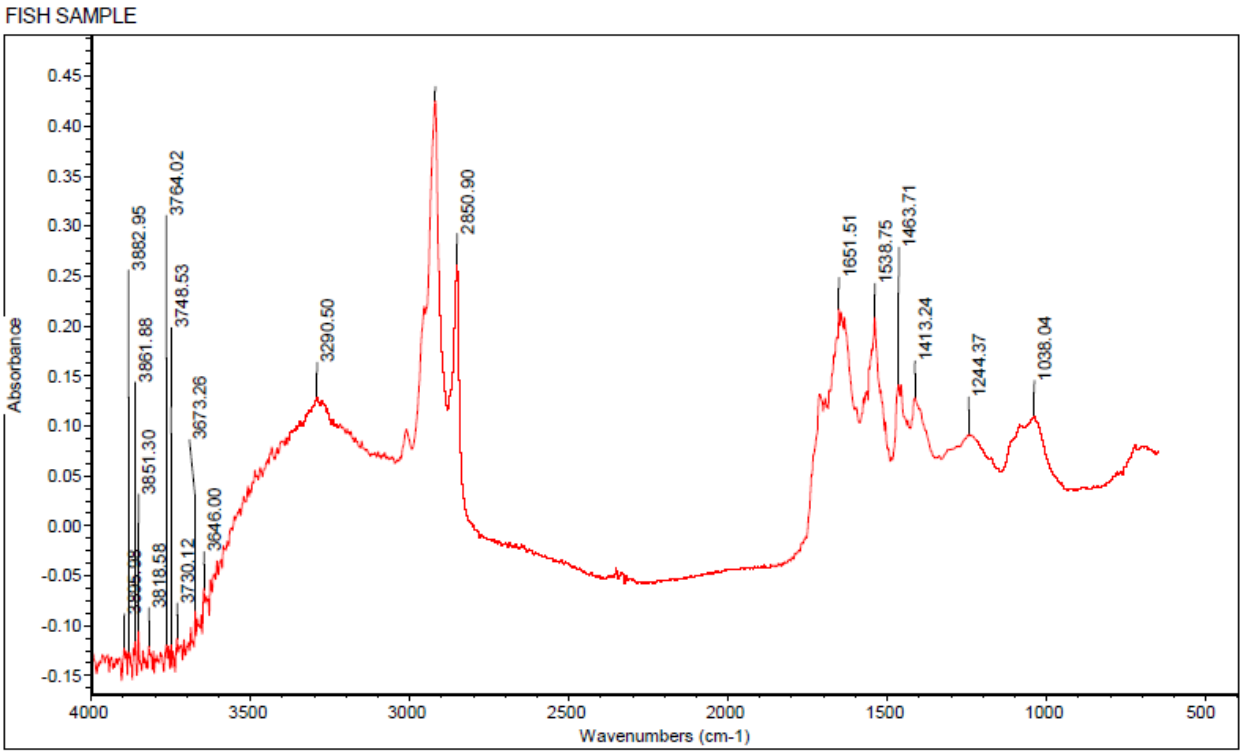


Fig . 2 FT-IR Analysis of Zebra fish mucus sample.

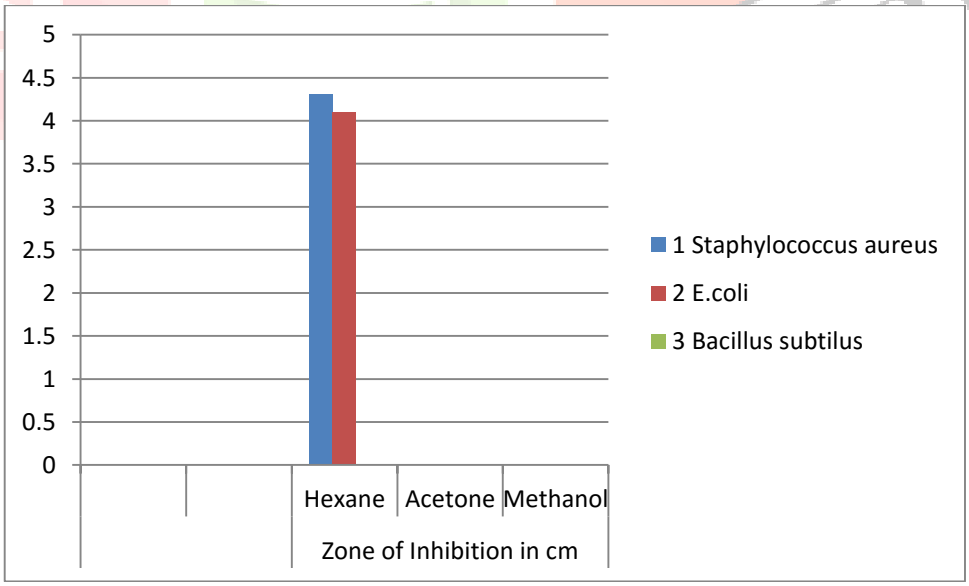


FIG – 3 GRAPH SHOWS THE ZONE OF INHIBITION IN CMS OF ZEBRAFISH MUCUS WITH THREE DIFFERENT SOLVENTS AGAINST THREE HUMAN PATHOGENS

ACKNOWLEDGMENT

I Would like thank Dr. Rajasekar SITRA Coimbatore for rendering help for all the work done in their lab.

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