ScienSage

Journal of Advanced Scientific Research

Available online through http://www.sciensage.info

ISSN
0976-9595
Short Communication

ISOLATION AND PARTIAL PURIFICATION OF ANTIBACTERIAL COMPOUND IN THE MUCUS OF THE ZEBRA FISH *DANIO RERIO*

Christy Shaila R*[1], Rini Joseph[1], Saminathan. K[2], Kathireswari. P[1]

[1]*Department of Zoology, Kongunadu College of Arts and science Coimbatore, Tamil Nadu*

[2]*Department of Chemistry, Kongunadu College of Arts and science, Coimbatore, Tamil Nadu*

**Corresponding author: christyshaila2178@gmail.com*

ABSTRACT

Synthetics from nature have been a part of human progress. The fish mucus is a protective secretion from the epidermal membranes of the surface which provides the first line of defense against pathogenic microbes. In the present study, a series of solvents such as hexane, acetone and methanol were utilized for extraction using thin layer chromatography for the isolation and partial purification of the antibacterial compound in the mucus of the zebra fish (*Danio rerio*). Among all the three solvents, the hexane solvent extraction alone indicated a slight yellow colour compound with Rf value 0.46 cm. Furthermore, these active compounds were confirmed with the Bio-autographic method by using two bacterial pathogens *viz., Staphylococcus aureus* and *E.coli* and obtained positive results. Therefore, this method is very convenient for searching chemical constituents with their biological activity, such as antibiotics.

Keywords: *Danio rerio* Mucus, Microbiological Screening, Antimicrobial, Thin Layer Chromatography, Bio autography.

1. INTRODUCTION

The zebra fish (*Danio rerio*) is a small, symmetrical tropical fresh water fish and omnivorous, they feed on zooplanktons. They are cold blooded animal and weigh about 2 to 3 gms. They can generate hundreds of progeny at daily intervals and they are nearly transparent and 84% of the genes are known to be associated with the humans so that they are considered as model organism [1].

Fishes live in an environment that is rich in microbes and are vulnerable to be invaded by pathogenic microorganisms. Over a very long period of time it is known that the mucus plays an important role in the colonization by parasites, bacteria and fungi. The fish skin or mucus which is used for research on some of the biologically active compound can be an interesting exercise [2]. Many of the antimicrobial macromolecules which are usually taken from the fish skin provide a first line barrier against the invasion of the pathogenic microbes. The mucus layer which covers the outer surface of the fish is said to have some mechanical protective functions. The antimicrobial peptides from the fish is said to be highly defensive against the pathogens than the amphibian skin. 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 [3].

At present, we are in need of safer, cheaper and effective drugs. The mucus of the zebra fish showed a high antibacterial activity against *Staphylococcus aureus* and *Escherichia coli* [4]. The potential of the zebra fish mucus as a source of biological products is largely unexplored in India. Hence, a broad screening of zebra fish mucus for isolation of bioactive compound is necessary and thin layer chromatography helps for analyzing and separating the compounds in the mixes [5].

2. MATERIAL AND METHODS

2.1. Collection of mucus from the zebra fish

Danio rerio was purchased from an aquarium in Coimbatore; the fishes were brought to the laboratory and acclimatized for a period of one week. Mucus was collected as described by Al-Hassan *et al.,* 1982 [6]. After one week mucus were collected from 12 adult fishes of average weighing about three grams each. Mucus was carefully scraped from the dorsal body surface using a sterile spatula and the mucus was not collected from the ventral side to avoid intestinal and sperm contamination. The mucus samples collected were lyophilized and stored under -20˚C for further analysis.

2.2. Thin-Layer Chromatography (TLC)

The sample extracts were applied to the thin- layer chromatographic plate with a capillary tube and placed in

developing chamber containing methanol as the developing solvent. Now the solvent is permitted to rise until it almost reaches the top of the plate which will give the maximum separation of the dye components [7]. TLC plate is then removed from developing chamber, dried and sprayed with Ninhydrin solution which showed slight yellow color spots when viewed under UV lamp.

2.3. Measurement of Rf values

The "retardation factor" (Rf value) were calculated by using the formula [8]:

$$Rf = \frac{\text{Distance moved by the solute}}{\text{Distance moved by the solvent}}$$

2.4. Thin layer chromatography by using bioautographic assay

The TLC Bioautography is used as a bioguiding method and it's convenient for searching substances with biological activity [9]. In the TLC of antimicrobials, a developed TLC plate was dipped in a suspension of microorganism growing in a nutrient broth and incubated in a humid atmosphere. The microorganism grows directly on the surface of the TLC plate, excluding the spots of antimicrobials. Visualization were done by spraying the plate with tetrazolium salt such as MTT (3-(4,5-dimethylthiazol-2)-2,5-diphenyltetrazolium bromide). The tetrazolium salt was converted into a creamy spot called the inhibition zones, by the action of the dehydrogenase of the living organisms, pointed to the presence of antimicrobial agents [10].The applications of the TLC-Bioautography tests were parallelly linked to spectroscopic methods helped to obtain the complete information of their bioactivity of the target compounds.

3. RESULTS

3.1. Thin layer Chromatography

Thin-layer chromatography profiling was done for the zebra fish mucus extract. In the present study, the mucus of the zebra fish along with three different solvents *viz*., Hexane, Acetone and Methanol were used for extraction by using thin layer chromatography. Among all solvents, the hexane solvent extraction alone indicated a slight yellow color compound with Rf value 0.46 cm and indicates the presence of amino acids and peptides. In the preliminary study the antibacterial activity of the zebra fish mucus was done against *Staphylococcus aureus, Escherichia coli* and *Bacillus subtilus* using hexane, acetone and methanol [4]. Among all the solvents maximum zone of inhibition was obtained for *Staphylococcus aureus* and *Escherichia coli* in the hexane solvent. The mucus of the Zebra fish along with color changes in the mobile phases with their Rf values were presented in Table 1. The movement of the components in the TLC plate was observed and its derivatizations are shown in Fig. 1.

Table 1: Thin layer chromatographic observations of zebra fish mucus by using different solvents

Solvent	Spraying Reagent	Visualization					
		Eyes			UV Lamp		
		Spot	Color	Rf	Spot	Color	Rf
Hexane	Ninhydrin	1	Pale Yellow	0.46	1	Yellow	0.46
Acetone	Ninhydrin	0	-	-	0	-	-
Methanol	Ninhydrin	0	-	-	0	-	-

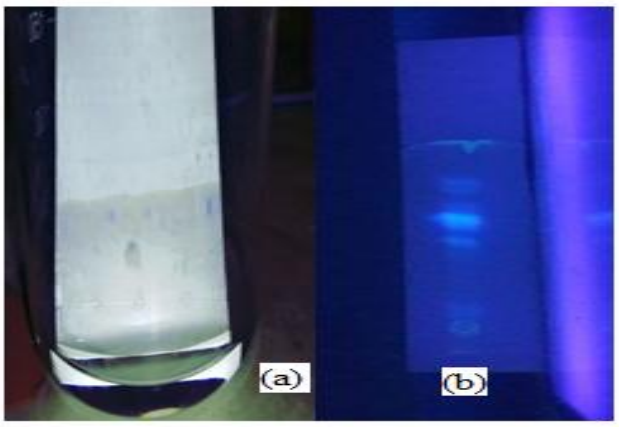

Fig.1: TLC plate (a) before derivitization (b) after derivitization

3.2. *In-vitro* antibacterial activity by bioautographic assay

In the present study it is revealed that the Rf values of the hexane solvent were higher. There was no significant variation for the other two solvents *viz*., acetone and methanol. Further to find out the particular compound and to study the bio-autography, the antibacterial activities of the hexane solvent extraction was investigated against human pathogenic bacteria such as *Staphylococcus aureus* (Gram positive), *Escherichia coli* (Gram negative). The hexane extract showed very high inhibitory activity against gram positive bacteria

Staphylococcus aureus (16.02±1.04 mm) and minimum inhibition of gram negative bacteria in *E. coli* was (11.07±1.02 mm). The zone of inhibition was measured and summarized in Table-2 and the evaluation of the TLC plate using *in vitro* antibacterial activity by Bio-autographic assay were shown in Figure 2 and 3.

Table 2: The evaluation of TLC plate by using the Bio - autographic assay

Treatments	Pathogenic organisms	Inhibition Zone (mm)
Control	Chloramphenicol	17.01±1.01
Gram Positive	*S. aureus*	16.02±1.04
Gram Negative	*E. coli*	11.07±1.02

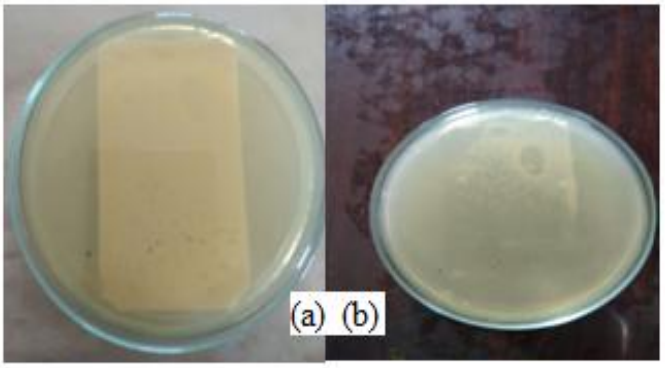

Fig. 2: The evaluation of bio-autographic assay in the zebra fish mucus using *In vitro* antibacterial activity using hexane solvent (*Staphylococcus aureus*) (a) TLC plate kept in a nutrient broth (b) Zone of inhibition developed on the TLC plate.

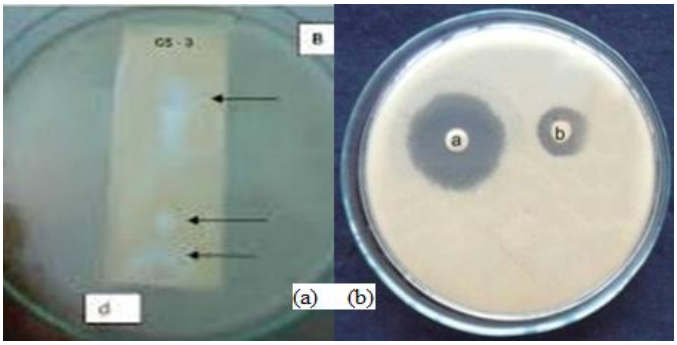

Fig. 3: The evaluation of bio-autographic assay in the zebra fish mucus using *in vitro* antibacterial activity by using hexane solvent (*Escherichia coli*) a) TLC plate kept in a nutrient broth b) Zone of inhibition developed on the TLC plate.

4. DISCUSSIONS

Natural products are important in human health care and they can be used as starting materials for semi-synthetic drugs. The biological interface between fish and their aqueous environment consists of mucus layer composed of biochemical diverse secretions from epidermal and epithelial cells [11]. In general, the fishes are having much biological significant chemical compositions so as to be used in pharmaceuticals, cosmetics and pesticide industries. TLC profiling of the hexane extract gives an impressive results and directing towards the indication and the presence of bioactive compounds. TLC is used for the identification, purity testing and determination of the concentration of active ingredients and drug preparations, process control in synthetic manufacturing processes [12]. Various pharmaceuticals have accepted TLC technique for the detection of impurity in a drug or chemical substances.

Various chemical constituents in the sample gave the Rf value in hexane solvent system. This variation in the Rf values of the chemical constituents provides a very important clue in understanding the polarity of the compound. This information helps in selection of appropriate solvent system for further separation of compound from these extracts [13].

In the present study, results showed that the gram positive bacteria *Staphylococcus aureus* and gram negative bacteria *Escherichia coli* much significant results with biological assay having a couple of potential applications. The span of the zone of restraint can be utilized as an unpleasant gauge of the measure of the inhibitor compound since; the sweep of the inhibitory zone is corresponding to the logarithm of the measure of the compound causing the hindrance. Perhaps the most common use of thin layer chromatography plate bioassays is to narrow the range of possible antimicrobial compounds in the fresh water of zebra fish extract. The present results of TLC plate of the autobiography evidently supported with earlier report by Isabelle *et al.*, 2014; Irena *et al.*, 2011 [14,15]. In TLC bio-autographic method on mucus of the zebra fish extract can be beneficial for detection and characterization of some antibacterial compounds.

The emergence of bacterial strains resistant to many currently used antibiotics make the need for fresh approaches to the treatment of several diseases. To solve this problem all over the world scientists is searching various living resources for lead compounds for the development of drugs against multidrug resistant

pathogens. Our present study clearly showed that TLC-Bio autography active substances are resistant against pathogenic organism such as *Staphylococcus aureus* and *Escherichia coli*. So, it has been strongly suggested that, the present study should be considered as a valuable source for biological active chemical constituents with potential medicinal values.

Despite the large number of antimicrobial peptides purified from various animal sources [16] relatively few have been isolated from epithelial surfaces of aquatic animals. Pleurocidin was one of the first antimicrobial peptide isolated from a Teleost fish consists of 25-amino acid peptide with a broad spectrum of activity and is expressed by the mucous cells of flounder skin [17, 18].

In spite of the wide employment of sophisticated chromatographic technique coupled with an online bio assays bio autography is still proving its worth as a simple and inexpensive tool for simultaneous chemical-biological screening of natural sources. For any natural product the separation process is not easy, and if separated the amount is very less in maximum cases, so it is necessary to develop a process which can detect and biological activity can also be measured successively. Considering all these we can say that bio autographic detection techniques would create a new era in the separation process [19].

It is important to realize that bio autography technique is not a quantitative measure of the antibacterial activity, but it indicates only the number of compounds that has to be separated by means of antibacterial activity [20]. The results showed that *Staphylococcus aureus* which has more inhibition bands does not mean that it was the most susceptible organism, but it might because some of the compounds are active only against that bacterium, when compared to other organisms selected for this study.

5. CONCLUSION

The present investigation reported that the mucus of the zebra fish has a remarkable antibacterial activity, which confirms that, the presence of some antimicrobial compound responsible for the invasion of pathogens. Further, the epidermal skin mucus of fish possess many bacterial substances which can be a potential source of novel antibacterial components in aqua culture practices and for the development of novel therapeutic agents to treat drug resistant pathogens and further in-depth investigations and structural elucidation is essential to isolate the compound.

6. ACKNOWLEDGEMENT

Authors would like express sincere thanks to Abbes Biotech laboratory for helping in carrying out the Bioautographic studies.

7. REFERENCES

1. Jason R Meyers. *Current Protocol Essential Laboratory Technique*, 2018; **16:**215-225.
2. Ebran N, Julien S, Orange N, Ausperin B, Molle G. *Biochimica et Biophysicas Acta*, 2000; **1467:**271-280.
3. Ellis AE. *Fish Immunology*, 1999; **20:**291-308.
4. Christy Shaila R, Kathireswari P. *International Journal of Creative Research Thoughts*, 2018; **6:**1335-1338.
5. Ali M, Agarwal V. *Separation Science and Technology*, 2002; **81:**363-377.
6. Al-hassan JM, Thompson M, Criddle RS. *Marine Biology*, 1982; **70:**27-33.
7. Austin B, Mc Intosh D. *Journal of Fish Diseases,* 1988; **11:**275-277.
8. Bipin D Lade, Anita S Patil, Hariprasad M Paikrao, Ankit S Kale, Kushal K Hire. *Research Journal of Pharmaceutical, Biological and Chemical Sciences*, 2005; **8:**115-128
9. Goodall RR, Levi AA. *Nature*, 1946; **158:**675-676.
10. Justus G, Kirchner. *Journal of Chromatography*, 1973; **82:**101-115.
11. Oren Z, Shai Y. *European Journal of Biochemistry*, 1996; **237:**303-310
12. Shephard KL. *Advanced drug delivery*, 1993; **11:**403-417.
13. Sanjeet Kumar, Jyotirmayee, Monalisa Sarangi. *International Journal of Pharmaceutical Sciences Rev.,* 2013; **18:**126-132.
14. Isabelle A, Kagan, Michael D Flynthe. *Journal of Immunology*, 2014; **173:**5626-5634.
15. Irena M. Choma, Edyta M. Grzelak. *Journal of Chromatography*, 2010; **1218:**2684-2691
16. Zasloff M Magainins. *Proceedings of the National Academic Sciences USA*, 1987; **84:**5449-5453
17. Cole AM, Darouiche R, Legarda D, Connell N, Diamond G. *Antimicrobial Agents Chemotherapy*, 2000; **44:**2039-2045.
18. Birkemo GA, Luders T, Anderson O, Nes IF, Nissen-Meyyer J. *Biochemical Biophysics Acta*, 2003; **1646:**207-215.
19. Cole AM, Weis P, Diamond G. *Journal of Biological Chemistry*, 1997; **272:**12008-12013.
20. Grinde B, Jolles J, Jolles P. *European Journal of Biochemistry*, 1988; **173:**269-273.