

Studies on the Antibacterial Activity of Bioactive Compounds of Fish *Tetraodon Fluviatilis* of West Coast of Mumbai

G.V. ZODAPE

Departments of Zoology, S.S. & L.S. Patkar College of Arts and Science & V.P. Varde College of Commerce and Economics, S.V. Road, Goregaon (West), Mumbai- 400 062, India.

*Corresponding author E-mail: drgautamvz5@gmail.com

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ABSTRACT

In the present investigation, an inhibition of bacterial growth noticed in pathogenic bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi*, and non-pathogenic bacteria *Escherichia coli*. The data on diameter (mm) of zone of inhibition of the crude extracts and isolated compounds from the whole body, liver, skin and remaining body tissues of *Tetraodon fluviatilis* on each strain of bacteria showed the trends in degree of zone of inhibition was also observed in the crude extracts and isolated compounds. It showed that the bacteria *Escherichia coli* > *Staphylococcus aureus* > *Streptococcus pyogenes* > *Pseudomonas aeruginosa* > *Klebsiella pneumoniae* > *Salmonella typhi*. The degree of zone of inhibition was observed in isolated compounds can also be put as II > III > IV. The range of inhibitory zone in bacterial cultures of isolated compounds observed from 6 mm (minimum) to 20 mm (maximum). Therefore, the biopotential activity of fish *Tetraodon fluviatilis* (fugu fish) found in the crude extract as well as in isolated compounds. The results of present investigation clearly confirm the antibacterial activity of the crude extract and isolated compounds from tissues of the fish *Tetraodon fluviatilis* (fugu fish) confirmed.

Keywords: *Tetraodon fluviatilis*, Antibacterial, Biopotential activity, Pathogenic, non-pathogenic bacteria.

INTRODUCTION

Modern technologies have opened vast areas of research for the extraction of biomedical compounds from ocean & seas, because of high percentage of biomedical compounds exhibit substantial pharmacological activity. During the last 30 years, the field of marine natural product chemistry has provided to be a prolific source of novel biologically active compounds. In recent years, collaboration between chemists, biochemists

& pharmacologists have seen the emergence of bioassay directed fractionation strategies as the most common route to the discovery of novel marine metabolites.

India has total length of the coastline is around 8014 Km which includes coastline of two groups of oceanic islands namely Andaman and Nicobar and Lakshadweep. India possess a vast Exclusive Economic Zone of 20,13,410 Sq. Km and territorial waters of 1,55889 Sq Km. The Indian coast



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has a variety of sensitive ecosystem like lagoons, sand dunes, coral reefs, mangroves, sea grass beds and wetlands ¹. Mumbai, the island city situated on west coast of India (between 18051' to 19033' N and long 72043' 73001' E). Arabian Sea enriches the Mumbai with a shoreline of 100 Km. the coastal areas in and around Mumbai are biologically most productive areas supporting a wealth of marine resources.

The Indian sub continent offers potential for pursuing marine biotechnology research for discovering novel biologically active compounds, which could be use in a large spectrum of human ailments and harvest bio resources for sustainable development with this context. Indian labs have concentrated on bioactive substances from marine animals such as horseshoe crab, green mussels, sponges and corals for characterization of novel molecules. The Department of Biotechnology (DBT) has been promoting marine biotechnology in India for the last one and half decade. Many R&D programs sponsored on marine biotechnology are leading towards products and process developments and development of a viable technology for the commercial production systems. The work carried out by NIO ² and others consist of bioactivity of the compounds collected from the coast of south and south – east India. The work has been reported by Venkateshvaran & Paniprasad ³ - CIFE Mumbai, & very scanty effort has gone into unraveling the details on the bioactive compounds by other

researchers. However, Zodape and Kulkarni ⁴, Zodape *et al.*,^{5, 6} have isolated and characterized the bioactive compounds from Intertidal crab *Leptodius exratus* collected from Nariman Point coast of west coast of Mumbai. They have also studied the antibacterial and pesticidal activities of the extract of *Leptodius exratus*. Zodaape and Berde ⁷ have studied the pharmacological activities like antibacterial, antifungal, pesticidal properties of the crude extract, and isolated compounds from (fugu fish) *Tetrodon oblongus* collected from west coast of Mumbai. Zodaape ⁸ isolated and characterized the bioactive compounds from intertidal crab *Atergatis integerrimus*. Zodaape ⁹, studied the antibacterial, antifungal and pesticidal activities of bioactive compounds of intertidal crab *Atergatis integerrimus* (Lamarck) of west coast of Mumbai and have concluded that, the Mumbai coast is under deterioration due to the presence of dinoflagellates and bacteria, which are eaten by the marine animals causing the presence of toxic compounds in the body of marine animals. Therefore, the present study has undertaken to explore the bioactive compounds from *Tetraodon fluviatilis* of Mumbai coasts and its extract investigated for the presence pesticidal activities.

MATERIALS AND METHODS

Method Of Collection

During low tide the specimens of fish was collected from Colaba (TIFR and NCPA) costal

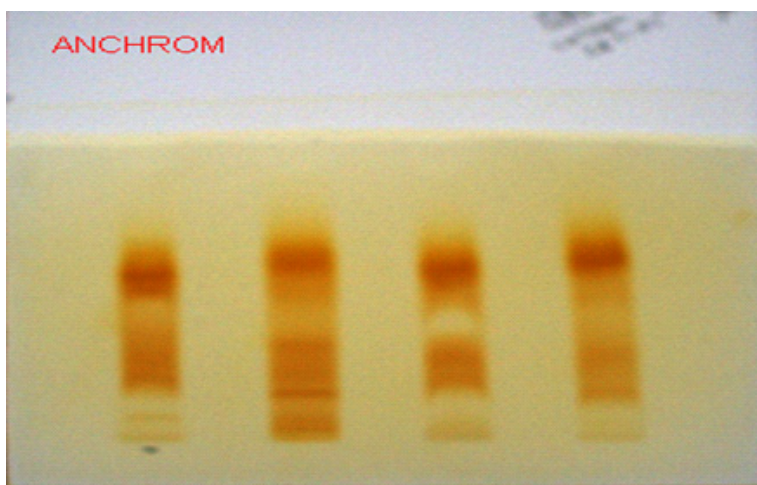


Fig. 1: TLC of crude extracts whole body, liver, skin and remaining body tissues showing four isolated compounds of fish *Tetraodon fluviatilis*

Table 1: Effect of the crude extracts and isolated compounds from Whole body, Liver, Skin and Remaining tissues of fish *Tetraodon fluviatilis* on bacteria

| | Diameters of zones of inhibition in mm | | | | | | | | | | | | | | | | | | | | | | | | |
|-------------------|--|----|----|----|----------------------|----|----|----|----------------|----|----|----|--------------------|----|----|----|----------------------|----|----|----|-----------------|----|----|----|----|
| | <i>S. aureus</i> | | | | <i>P. aeruginosa</i> | | | | <i>E. coli</i> | | | | <i>S. pyogenes</i> | | | | <i>K. pneumoniae</i> | | | | <i>S. typhi</i> | | | | |
| | Cr. | C. | C. | EX | Cr. | C. | C. | EX | Cr. | C. | C. | EX | Cr. | C. | C. | EX | Cr. | C. | C. | EX | Cr. | C. | C. | EX | |
| Whole body | 14 | 13 | 15 | 12 | 10 | 13 | 12 | 14 | 11 | 11 | 12 | 11 | 14 | 13 | 09 | 11 | 10 | 13 | 12 | 08 | 10 | 09 | 12 | 11 | 07 |
| Liver | 18 | 15 | 19 | 16 | 12 | 17 | 14 | 18 | 15 | 13 | 19 | 13 | 18 | 17 | 11 | 16 | 12 | 17 | 16 | 10 | 15 | 11 | 16 | 15 | 09 |
| Skin | 16 | 14 | 18 | 15 | 12 | 15 | 13 | 17 | 14 | 13 | 17 | 12 | 17 | 16 | 11 | 16 | 11 | 16 | 15 | 10 | 15 | 10 | 15 | 14 | 09 |
| Remaining tissues | 13 | 11 | 10 | 19 | 09 | 14 | 10 | 09 | 09 | 10 | 15 | 09 | 11 | 11 | 08 | 11 | 08 | 11 | 09 | 07 | 10 | 07 | 10 | 06 | 06 |
| Control | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 | 00 |

Cr.CX-Crude Extract; Isolated Compound, I- C.I Isolated Compound, II- C.II Isolated Compound, III- C.III Isolated Compound, VI- C.IV Isolated from Whole body, Liver, Skin and Remaining tissues

area. After collection, the fishes transported for acclimatization in glass aquarium (12 x 12 x 18 cm.) containing seawater collected from the site to the laboratory of Patkar College Goregaon (west), Mumbai during the monsoon season and acclimatized for 2 days at room temperature before use.

Identification Of Fish

The identification of the fishes was done at The Central Institute Of Fishery Research (CIFE) Versova Andheri Mimbai.

Preparation Of Crude Extract

After acclimatization, live fishes were selected and whole body, skin, liver and other body tissues dissected out. The dissected parts were weight and crushed separately in an equal volume of a mixture of methanol and acetic acid (w/v). Finely crushed fishes homogenized with a mixture of 80% methanol and 1% acetic acid by heating in water bath at 50°C for half an hrs. The process repeated with more amount of methanol-acetic acid mixture (5ml) thrice. The supernatant solution decanted off and centrifuged at 3000 rpm for 20 minutes. The residue settled if any rejected and the clear supernatant solution placed in a separating funnel and extracted with dichloromethane to de-fat the solution. The upper clear de-fated aqueous solution was taken in a beaker and heated on a water bath 40-45°C till solid obtained. This solid weighed and dissolved in 1% aqueous Tween- 80 solution such that the concentration of the solution corresponds to 1mg/mL and stored in screw-capped vials in a refrigerator at -20°C until further use ¹⁰.

Extraction Or Isolation Of Bioactive Compounds

HPTLC performed on 'CAMAG TLC' system at Ancrom test lab Mulund (E) Mumbai. The stationary phase was pre coated Aluminium plates with a silica gel (₆₀ F²⁵⁴), and the mobile phase was butanol: methanol: water (3:1:1). Densitometric scanning performed at 254nm using Deutorium lamp. The development of spot was done using twin trough chamber. Preparative chromatographic separation was carried out using 0.5mm thick stationary phase and 5ml of the crude extract was spotted on a preparative TLC and dried and kept in the saturated twin trough chamber containing butanol :methanol: water in the ratio of 3:1:1(v/v) as

a mobile phase and developed up to 9 cm length . The plate removed and dried. The development of the sample spots were done using twin trough chamber. The Deutorium lamp at 254 nm used for densitometric scanning of the samples. The four spots obtained. The spots scrapped off, dissolved in methanol, filtered, and evaporated to dryness to get pure compounds.

All chemicals and solvents used were of analytical grade supplied by M/S S.D. fine chemicals, Thane, (India).

Collection Of The Animals For Evaluating Effects Of The Extracts

To assay bioactivity of the crude and isolated extracts, their effect on bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, *Psuedomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi* was studied by using standard microbiological methods.

Protocol for evaluating effect of crab extracts on the microbes

The pure culture of bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, *Psuedomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi* were collected from The Department Of Microbiology Mithibai College Vile Parle, Mumbai. The culture was stored in a refrigerator at 2-8 °C.

The nutrient media prepared by dissolving 5g of peptone, 3g beef extract, 8g sodium chloride and 150g agar in about 800ml of water and adjusting the pH of the solutions to 7.3 by drop wise addition of 1N sodium hydroxide. The solution heated 2-3 minutes, cooled and diluted to 1litre with distilled water. All the apparatus such as syringes, pipettes, conical flasks, Petridishes and the nutrient media sterilized in an autoclave before their use. A basic culture medium used for growing bacterial culture under laboratory condition ¹¹.

The pure culture of bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, *Psuedomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi* were spread in sterile petridishes by streaking method.

Total Twelve petridishes were prepared. In all the petridishes, the crude extract of whole body, liver, skin and remaining body tissues was added on marked areas of the first six petridishes by putting a whatman filter paper No. 40 (5 mm diameter) on the marked area of each of the petridish . 0.1 ml of each of the crude extract placed on filter paper by injection vile. The same procedure used by adding 10 µL for the isolated compounds. Control also maintained by adding methanol to the respective petridishes. Then petridishes kept for incubation for 24 hours at 37°C.

RESULTS AND DISCUSSION

Effect of the crude extracts and isolated compounds of fish *Tetraodon fluviatilis* on bacteria

The effect of the crude extract and isolated compounds from of whole body, liver, skin and remaining body tissues of *Tetraodon fluviatilis* on bacteria *Staphylococcus aureus*, *Streptococcus pyogenes*, *Psuedomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi* is presented in Table. No. 1. It is evident from the photograph that the extract inhibited the growth of bacteria cultured in the petridishes. The inhibition of growth of bacteria calculated in terms of zones of inhibition observed in each petridish of size 9 x 2 cms. The photographs showed the effect of the isolated compounds from the whole body, liver, skin and remaining body tissues on bacteria (*Staphylococcus aureus*, *Streptococcus pyogenes*, *Psuedomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Salmonella typhi*). A maximum inhibitory zone developed after adding crude extract and isolated compounds from the whole body, liver, skin and remaining body tissues.

Table No. (1) Represents the data on diameter (mm) of zone of inhibition of crude extract of *Tetraodon fluviatilis* from the whole body, liver, skin and remaining body tissues on each strain of bacteria. It was evident from the table that a maximum inhibition observed in the petridish of *Escherichia coli* culture whereas, in case of *Salmonella typhi* it showed minimum zone of inhibition in remaining body tissues. It also observed that the maximum inhibition observed in liver as compared to the skin, whole body and remaining

body tissues respectively. The range of inhibitory zone in bacterial cultures observed from 06 mm (minimum) to 20 mm (maximum).

Table No. (1.) Also represents the data on diameter (mm) of zone of inhibition of the isolated compounds of *Tetraodon fluviatilis* from the whole body, liver, skin and remaining body tissues on each strain of bacteria. From the table it was evident that the compounds isolated from the liver shows maximum zones of inhibition in compound II as compared to the compound III compound I and compound IV. It was also evident from the table that the compounds isolated from the liver extract showed maximum zones if inhibition than the compounds extracted from skin, whole body and remaining body tissues. The trends in degree of zone of inhibition was also observed in the isolated compounds from whole body, liver, skin and remaining body tissues on bacteria can be put as bacteria *Escherichia coli* > *Staphylococcus aureus* > *Streptococcus pyogenes* > *Psuedomonas aeruginosa* > *Klebsiella pneumoniae* > *Salmonella typhi*. The degree of zone of inhibition was observed in isolated compounds can also be put as II > III > IV. The range of inhibitory zone in bacterial cultures of isolated compounds observed from 6 mm (minimum) to 20 mm (maximum).

CONCLUSION

In the present investigation, an inhibition of bacterial growth noticed in pathogenic bacteria *Staphylococcus aureus* *Streptococcus pyogenes*, *Psuedomonas aeruginosa*, *Klebsiella pneumoniae*, *Salmonella typhi* and *non-pathogenic bacteria Escherichia coli*. Therefore, it confirmed that the biopotential activity found in the crude extract as well as in isolated compounds of fish *Tetraodon fluviatilis* (fugu fish). The activity expressed in terms of zone of inhibition. Since substantial inhibition of growth of bacteria observed, presence of toxin in crude and isolated extracts of fish *Tetraodon fluviatilis* (fugu fish). The results of present investigation clearly confirm the antibacterial activity of the crude extract and isolated compounds from tissues of the fish *Tetraodon fluviatilis* (fugu fish). In conclusion, a presence of toxins in crude extracts and isolated compounds from the whole body, liver, skin and remaining body tissues of fish *Tetraodon fluviatilis* confirmed.

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