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Original Research Article

Antimicrobial and Antifungal Activity with Special Reference to Anti-Mycobacterium Tuberculosis Activity of Puffer Fish *Lagocephalus Spadiceus* (Gmelin, 1789) Collected from Arnala Beach Virar, West Coast of Mumbai, India

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Abstract

Background: This study aims to investigate the antibacterial and antifungal properties against the crude extract of liver, skin and intestine of Puffer fish *Lgocephalus spediceous*. The study specifically focuses on the crude extract and its efficacy on antibacterial, antifungal, and anti-mycobacterial activity. The Puffer fish *Lagocephalus spadicoeus* were collected during low tides from Arnala, Virar, West Coast of Mumbai. The liver, skin and intestine were dissected out separately. The dissected parts were weighed and were crushed separately in an equal volume of a mixture of 80 % methanol and 1 % acetic acid (w/v) for 24 hours in the water bath at 45º C. The homogenates were centrifuged at 10,000 rpm for 20 minutes in cold centrifuge at -8ºC and supernatant was collected. The supernatant aliquots were concentrated in a rotary vacuum evaporator at 45º C. The resultant extracts of liver, skin and intestine were subjected to Millipore filter system, dried in vacuum desiccator, and used for anti-bacterial and antifungal activity. From the above results it was confirms that the crude extract of liver of puffer fish *L. spadecius* showed more efficacy than the skin and intestine. The zones of inhibitions measured as Liver > skin > intestine. Whereas no antifungal activity was noted against both the fungal strains *C. albicans* ATCC 10231 and *C. tropicalis.* The sensitivity of crude extract of puffer fish *L. spadecius* against *M. tuberculosis* was found in skin, 6.25µg/ml, in liver 100µg/ml, and in intestine, 100µg/ml respectively. From the above results, it is found that the sensitivity of crud extract of *L. spadecius* measured in terms of sensitivity as skin > liver =intestine. From the above results it was found that of puffer fish *L. spadecius* have strong antibacterial and anti-mycobacterial property.

Keywords: Puffer fish, Crude extract, Anti-bacterial, anti-fungal, Anti-tubercular property.

Introduction

Marine environment has played a crucial role for the origin of lead molecules for various pharmacological utilizations in recent times. Interestingly marine organism's remains the most unexplored and essential provenience of umpteen bioactive metabolites. Generally, 70% of the biosphere organisms are found in inshore to abysmal sea waters because of the varying temperature, pressure, and source of light in the marine system [1]. Since the beginning of the mankind, marine natural products have been used for human aliments. The ocean covers more than three-quarters of the earth surface and harbor most of the planet's diversity. Marine microbial natural products have been the reservoir for drug discovery, yet the microorganisms inhabiting the world's oceans have largely been overlooked in this regard [2]. Marine organisms have a shorter history of utilization in the treatment and prevention of human disease. Then, drug discovery research from marine organisms is accelerating and now involves interdisciplinary research including biochemistry, biology, ecology, organic chemistry and pharmacology [3,4]. Thousands of new marine natural products have been reported, belonging to chemical classes of steroids, terpenoids, isoprenoids, nonisoprenoids, quinones, brominated compounds, nitrogen heterocyclics, and nitrogen sulphur heterocyclics. Many new developments of the last five years in this field of research and important findings for bioactive compounds from in vitro, in vivo and clinical studies for therapeutic drug applications have been covered [5]. Chemical substances derived from animals, plants and microorganisms have been used to treat human diseases since the dawn of medicine. The investigation of these chemical substances from natural products as source of human therapeutics

reached its peak in the period 1970–1980, which resulted in strong influence of non-synthetic molecules to pharmaceutical industries.

In India, an exponential abundance of puffer fishes has been in Arabian Sea since 2006. Nearly 2000 toons of these fishes were recorded in 2011. These fishes have gained high attention as a new fishery resource [6]. Puffer fishes are toxic, even though they are considering as delicacy in many countries especially Japan and others [7]. In Japan, nearly 20 to 100 persons pass away every year because of puffer fish poisoning [8]. These countries investigate the food source and gut content analysis of puffer fishes. In India the first instance fish poisoning was reported due to consuming the fried puffer fish *Chelenodon patocaroe* led to a number of fatalities [9]. In India, utilization of puffer liver as a source of fish oil and its polyunsaturated fatty acids are used for human aliments. These fish provide the best source of raw material for collagen extraction because of its high availability, no risk of disease transmission, no religious barriers and possibility of higher yielding collagen [10].

Studies on bioactive compounds are being extensively studied in Japan, China & other western countries, but in the developing countries like India, Marine organisms have been explored for the isolation but have not been explored for its structural elucidation. In India, many research organizations like Marine Products Export Development Authority (MPEDA) working on antibiotics in aquaculture, particularly in shrimp farming [11]. National Institute of Oceanography (NIO) in collaboration with Central Drug Research Institute (CDRI), Lucknow, has taken a program on "Development of Potential Drugs from the seas around India". Advanced Centre for treatment, research and education in Cancer (ACTREC), Indian Institute of Chemical Technology (IICT), Hyderabad, and ten other laboratories are working on bioactive compounds present in marine organisms [12]. The Ministry of Earth Sciences (MoES), New Delhi, supports this program and under this program, therapeutic potential of several isolated and identified compounds have been explored and there are hopes of few of the lead compounds identified reaching the drug stage. Several new compounds from marine origin are now under clinical trials for drug development. Mumbai island city is located off the west coast of India (between longitude 18051' and 19033' N and long 72043' and 73001' E). The Arabian Sea blesses Mumbai with a 100 km long coastline. Mumbai island city is located off the west coast of India (between longitude 18051' and 19033' N and long 72043' and 73001' E) [13]. The Arabian Sea blesses Mumbai with a 100 km long coastline. The study carried out by Zodape and others have extensively explored the marine coast in and around Mumbai. They studied the Jelly Fish, Corals, Sponges, Puffer fish, and crabs collected from the marine west coast off Mumbai, India, and studies its antimicrobial, antifungal, Pesticidal, anti- mycobacterium and biomedical studies. They studied the antibacterial and antimycobacterial property of crude extract of box jelly fish *Chiropsoides buitendijki* (horstr, 1907) from west coast of Mumbai [14], biomedical activities of marine sponge *Suberites carnosus* (Johnston) collected from west coast of Mumbai, India [15], bioactive compounds from sponge *Suberites carnosus* (Johnston) collected from west coast of Mumbai, India [16], biomedical activities of marine sponge *Sigmadocia fibulata* (Schmidt) collected from west coast of Mumbai, India [17], antibacterial and pesticidal activity of marine sponges *Sigmadocia fibulata* (Schmidt) and *Suberites carnosus* (Johnston) collected from west coast of Mumbai, India [18], studies on the antibacterial activity of bioactive compounds of fish *Tetraodon fluviatilis* of west coast of Mumbai [19], studies on the antibacterial and antifungal activities of bioactive compounds of intertidal crab *Atergatis integerrimus* (Lamark) of west coast of Mumbai [20], biopotential activity of the extract isolated from intertidal crab *Leptodius exaratus* from Mumbai Nariman point coast [21] showed antibacterial, antifungal, pesticidal, anti-mycobacterium and biomedical properties. The coastal areas in and around Mumbai are biologically productive and support abundant marine resources [22].

Therefore, the present study has undertaken to explore the bioactive compounds from puffer fish *L.spadiceous* of Mumbai coasts and its extract investigated for the presence antibacterial, antifungal and anti-mycobacterium activities.

Materials and Methods

a) Sample Collection

The Puffer fish *Lagocephalus spadicoeus* were collected during low tides from Arnala Beach, virar, West Coast of Mumbai. Animals were taken alive to the laboratory in sea water washed under seawater and then with distilled water and the deep freezer at -20 º C at the Department of Zoology, Patkar -Varde College, Goregaon West, Mumbai.

b) Identification of Puffer Fish

Preliminary identification was done by examining the color, shape and size and by reviewing the literature Dr. Ramkumar, scientist, at the Central Marine Fisheries Research Institute (CMFRI), Mumbai, confirmed identification.

c) Preparation of crude extract

The fish sample was removed from the deep fridge, blotted with blotting paper and acclimatized at

room temperature for half an hour. The liver, skin and intestine were dissected out separately.

The dissected parts were weighed and were crushed separately in an equal volume of a mixture of 80 % methanol and 1 % acetic acid (w/v) for 24 hours in the water bath at 45º C. The procedure was followed thrice by adding the mixture of 80 % methanol and 1 % acetic acid intermittently. The resultant aliquot mixture or liver, skin and intestine obtained were filtered separately through Whatman filter paper No. 1.

The homogenates were centrifuged at 10,000 rpm for 20 minutes in cold centrifuge (Remi centrifuge serial No. VCDX- 5983) at -8ºC and supernatant was collected. The supernatant aliquots were concentrated in a rotary vacuum evaporator at 45º C. The resultant extracts of liver, skin and intestine were subjected to Millipore filter system, dried in vacuum desiccators, and stored in the refrigerator at – 20ºC until further use.

d) Ethical Approval

Ethical approval was sought from Maharashtra State Biodiversity Board, Nagpur, Maharashtra (No.: MSBB/Desk-5/ /Research/ 842/2022-23) for collection of Puffer fish samples for research purpose.

e) Procurement of bacterial and fungal cultures

The pure culture of bacteria strains *S. aureus ATCC 6538, S. mutans ATCC 25175, E.coli ATCC10536 , K. pneumonia ATCC 4352, Salmonella typhi ATCC 13311, Sarcina ventriculii ATCC 1842, Pseudomonas aeruginosa ATCC 15442, Corynebacterium diphtheria ATCC 13812,* and fungus *Candida albicans ATCC 10231, Candida tropicalis ATCC1923* and *Mycobacterium tuberculosis* (Vaccine strain, H37 RV) ATCC No-27294 was purchased and procured from the Central Research Laboratory, Maratha Mandal's NGH Institute of Dental Sciences and Research Centre, R.S. No. 47A/2, Bauxite Road, Belgaum, India.

f) Antibacterial and Antifungal Study

The Anti-bacterial and anti-fungal activity of crude extracts of liver, skin and intestine of *L. spadiceous* was assess by using the Brain Heart Infusion (BHI) agar disc diffusion method was used for anti-bacterial and Sabouraud agar disc diffusion medium was used as proposed by Kirby Bauer's disc diffusion method [23] and the anti-mycobacterial assay was evaluated by using Microplate Alamar Blue Assay (MABA) as proposed by [24].

Results and Discussion

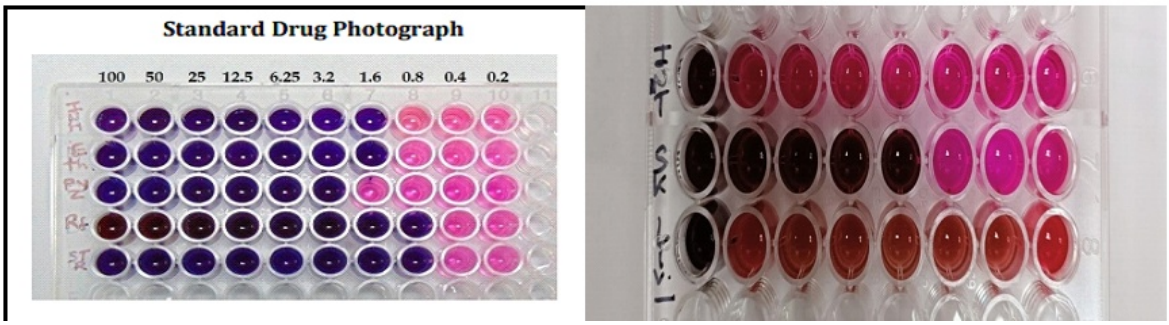
Table 1: Showing effect of crude extract of liver, skin and intestine of L *lagocephalus spadecius* (Gmelin, 1789) showing zone of inhibition against bacterial and fungal strains

Sr. No	Name of bacteria/fungus	Zone of Inhibition(Diameter in mm)		
		Skin	Liver	Intestine
1	*S. aureus ATCC 6538*	10mm	16mm	R
2	*S. mutans ATCC 25175*	R	10mm	R
3	*E.coli ATCC10536*	10mm	18mm	10mm
4	K. pneumonia ATCC 4352	9mm	15mm	7mm
5	*Salmonella typhi ATCC 13311*	12mm	23mm	10mm
6	*Sarcina ventriculii ATCC 1842*	10mm	14mm	5mm
7	*Pseudomonas* aeruginosa ATCC 15442	R	14mm	R
8	Corynebacterium diphtheria ATCC 13812	10mm	16mm	5mm
9	*Candida albicans ATCC 10231*	R	R	R
10	*Candida tropicalis ATCC 1923*	R	R	R

Table 2: Showing effect of methanolic crude extract of liver, skin and intenstion of *Lagocephalus spadecius* (Gmelin, 1789) on standard drugs on m. *tuberculosis strain* h37 rv: atcc no-- 27294 using Microplate Alamar Blue Assay (MABA)

Sr. No.	Sample	100 µg/ml	50 µg/ml	25 µg/ml	12.5 µg/ml	6.25 µg/ml	3.12 µg/ml	1.6 µg/ml	0.8 µg/ml
1	Skin	S	S	S	S	S	R	R	R
2	Liver	S	R	R	R	R	R	R	R
3	Intestine	S	R	R	R	R	R	R	R
5	Isoniazid	S	S	S	S	S	S	S	R
6	Ethambutol	S	S	S	S	S	S	S	R
7	Pyrazinamide	S	S	S	S	S	S	R	R
8	Rifampicin	S	S	S	S	S	S	S	S
9	Streptomycin	S	S	S	S	S	S	S	S

* S= Sensitive *R=Resistant



(A) **(B)**

Figure 1: **Photograph showing, A) Standard drug concentration on *M.tuberculosis.* B) Effect of crude extract of puffer fish *L. spadiceouson M. tuberculosis***

The antimicrobial activity of different tissues viz. skin, muscle, intestine and liver prepared in acetic acid extracts of puffer fish *A.stellatuss* showed antibacterial activity. It was observed that, liver extract exhibited strong (7mm diameter) against *K.pneumonia* [25]. The skin extracts of puffer fish *L. sceleratus* showed maximum activity against *Enterococcus faecalis* and *Bacillus subtilis* (gram positive bacteria), *Escherichia coli* and *Aeromonas veronii* (gram negative bacteria), whereas minimum activity was recorded against *Streptococcus agalactiae* (gram positive bacteria) and *Vibiro cholerae* (gram negative bacteria). In respect of antifungal activity, the maximum activity was observed against *Aspergillus fumigatus* and *Candida albicans* where the minimum activity was noted against *Trichophyton rubrum* by the skin extract of puffer fish *L. sceleratus* [26]. The in vitro antibacterial and antifungal activities of different tissue extract of the puffer fish *Takifugu oblongus* were screened against various antibacterial, antifungal by well diffusion method and larvicidal activity was accessed by analyzing the rate of mortality. The measured zones of inhibition of various tissue extracts were analyzed and found that those gonads extract possessed highly commendable antibacterial activity against most bacterial strains whereas lowest antibacterial activity noted against muscle extract and no antifungal activity was observed against both the e extracts. In case of larvicidal activity all the tissue extracts showed produced significant larvicidal activities. The maximum activity was noticed with skin extract, whereas muscle extract showed the least activity of *Takifugu oblongus* [27]. The antibacterial activity of six puffer fishes *Cyclichthys orbicularis, Diodon holocanthus, Canthigaster solandri, Arthron hispidus, A. inermis* and *Lagocephalua inermis (L. inermis)* were exhibited against 10 different human bacterial pathogens. It was observed that, *Arthron hispidus* showed maximum zone of inhibition (8 mm) against *Staphylococcus aureus* while *L. inermis* showed minimum activity (1 mm) against *P. mirabilis* and no zone of inhibition was observed against *S. aureus* [28]. The crude skin extracts prepared using 0.1% acetic acid of Puffer fish, *Arothron stellatus* showed inhibition activity against the bacterial and fungal strains. It was found that maximum activity was noted against *E. coli* and *S. aureus* and minimum activity against *K. pneumoniae,* in case of fungal strains maximum activity observed in *A. niger* and *A. flavus* and minimum activity against *T. viridae* [29]. Reports revealed that antimicrobial activity of skin acetic acid extract of puffer fish *A. stellatus* has shown activity against various bacterial and fungal organisms. The antibacterial activity was found maximum against *E.coli* and *S.aureus* and minimum against *K. pneumoniae,* antifungal activity was observed maximum against *A.nigar* and *A.flavus* and minimum activity against *T.viridae* [30]. The liver, skin, and muscle extract of puffer fish *A. hispidus* prepared in acetic acid showed antibacterial and antifungal property. It was observed that, skin extract shown maximum zone against the *E. coli* and *A.niger* and the liver extract has shown minimum against *P. vulgaris* and *T. viridae* [31]. The crude tissue extract of puffer fish *A. hispidus* were screened against seven human pathogenic bacteria for testing their antibacterial activities and the inhibition zones of the extracts were compared with standard ampicillin for bacterial culture. They observed maximum zones against *E. coli* in the skin extracts of *A. hispidus* and the minimum zones was observed against *P. vulgaris* in the liver extracts [32]. The tissue extracts of *Arothron hispidus* showed antimicrobial activity against the bacterial and fungal strains. It showed maximum activity against of *E.coli* by skin extract and minimum was found against *P.vulgaris* in liver extract [33]. The extract of the puffer fish *A.hispidus* produced strong activity against *A.niger* in the skin extract and the minimum was observed against *T. rubrum* in the liver extract with zone of inhibition 11.1mm [34]. The in vitro antibacterial and antifungal property was screened on ten human pathogenic bacteria and fungus against puffer fish

Arothron immaculatus by using its skin and liver extracts were subjected by standard disc diffusion method. The results confirmed a positive test against the pathogens used. From the results obtained, it showed maximum antimicrobial effect against *S. aureus* of 2.5 mm in liver extract and 9.8 mm against *V. cholera* in skin extract, whereas no no antifungal effect was noted against liver and extract of *Arothron immaculatus* [35] .The methonolic extract of puffer fish *A. calamus* had no activity against *Enterococus sp.* and *P. aeruginosa,* but in case of other human bacterial pathogens it showed inhibition zone ranged from 1-8 mm.[36] .It was reported that epithelial tissues of puffer fish *L. sceleratus* produce antimicrobial molecules which serve as the first line of a host defense against microbial invasion in vertebrates [37].

Table-1 shows the effect of crude extract of liver, skin, and intestine of puffer fish *L. spadecius* (Gmelin, 1789) on different bacterial and fungal strains by using (Hudzicki 2009) Kirby Bauer's disc diffusion method. It observed that puffer fish L. *spadecius* showed zone of inhibition against all bacterial strains *S. aureus ATCC 6538, S. mutans ATCC 25175, E.coli ATCC10536 , K. pneumonia ATCC 4352, S.typhi ATCC 13311, S. ventriculii ATCC 1842, P. aeruginosa ATCC 15442, C. diphtheria ATCC 13812.* Whereas no antifungal activity was noted against both the fungal strains *C. albicans* ATCC 10231 and *C. tropicalis* ATCC 1923. In the case of bacteria, *S. aureus* ATCC 6538 showed antibacterial activity in skin (10mm) and liver (16mm) whereas no activity was noted in crude extract of intestine. In *S. mutans* ATCC 25175 only zone of inhabitation was noted in liver extract (10mm) and no effect was observed in skin and intestine. *In E.coli* ATCC10536, *K. pneumonia* ATCC 4352, *S. typhi* ATCC 13311, *S. ventriculii* ATCC 1842, and *C. diphtheria* ATCC 13812 all the extracts, skin, liver, and intestine showed positive effect is in the form of zones of inhibitions. In *P. aeruginosa* ATCC 15442 only liver shows zone of inhibition (14mm), whereas no effect was noted in skin and intestine extract. From the above results it was observed that the maximum zone of inhibition was noted in liver extract of *E.coli* ATCC10536 (18mm), where minimum zone of inhibition was observed in liver extract of S. mutans ATCC 25175 (10mm). In the case of crude extract of skin of *L. spadecius,* the maximum and minimum activity was found in *S. typhi* ATCC 13311 (12mm) and *K. pneumonia* ATCC 4352 (9mm) respectively, whereas no activity was noted in *S. mutans* ATCC 25175 and *P. aeruginosa* ATCC 15442. In skin extract of *L. spadecius* (Gmelin, 1789) the maximum zone of inhibition was noted in *E.coli* ATCC10536 and *S. typhi* ATCC 13311 (10mm) respectively and minimum zone of inhibition was noted in *S ventriculii* ATCC 1842 (5mm) and no zone of inhibition was noted in *S. aureus* ATCC 6538, *S. mutans* ATCC 25175 and *P. aeruginosa* ATCC 15442. From the above results it was confirms that the crude extract of liver of puffer fish *L. spadecius* showed more efficacy than the skin and intestine. The zones of inhibitions measured as Liver > skin > intestine.

Table-2 and photograph 1 (A&B), shows the effect of crude extract of liver, skin, and intestine of puffer fish *L. spadecius* on *M. tuberculosisstrain* H37 Rv: ATCC No.27294.The results were compared with the standard drugs using microplat ealamar blue assay (MABA). Fig. 1 showing the effect of standard drugs and crude extract of liver, skin, and intestine of puffer fish *L. spadecius* on *M..tuberculosis*. The sensitivity of standard drugs against *M. tuberculosis* was noted as Isoniazid (1.6 µg/ml), Ethambutol (1.6 µg/ml), Pyrazinamide (3.125µg/ml), Rifampicin (0.8µg/ml), and Streptomycin (0.8µg/ml) respectively. The sensitivity of crude extract of puffer fish *L. spadecius* against *M. tuberculosis* was noted skin (6.25 µg/ml), liver (100 µg/ml) and intestine (100µg/ml) respectively. From the above results, it is found that the sensitivity of crud extract of *L. spadecius* measured in terms of sensitivity as skin > liver =intestine. From the above results it was confirmed that the crude extract of crude extract of puffer fish *L. spadecius* contains bioactive compounds which showed anti-bacterial and anti-mycobacterial property whereas both the fungal strains *C. albicans* ATCC 10231 and *C. tropicalis* ATCC 1923 were found to be resistant to the crude extract of puffer fish *L. spadecius*. The results of this study on antibacterial and antifungal are in the agreement with the results cited here in above. However, this study showed novelty against Mycobacterium tuberculosis. So far date no studies have been revealed on anti-mycobacterial activity against crude extract of puffer fish *L. spadecius*. From this study it confirms that, the crude extract of crude extract of puffer fish *L. spadecius* showed efficacy against *M. tuberculosis* strain. So it is suggested that in the future puffer fish *L. spadecius* may be used as an effective antibacterial and anti-mycobacterial agent.

Conclusion

The present study concluded the effect of crude extract of of puffer fish *L. spadecius*. on different bacterial and fungal strains. From the above results it was found that of puffer fish *L. spadecius* have strong antibacterial and anti-mycobacterial property. It also confirms that the crude extract of puffer fish *L. spadecius* has drug sensitivity against anti-mycobacterial drugs. Thus the study on puffer fish *L. spadecius* highlights the significant role that it may be useful for the development of new drugs as it has extraordinary antibacterial, and anti-tuberculosis effects, underscore the potential of these organisms as a rich source of bioactive

compounds. The crude extract of puffer fish *L. spadecius* will be processed further for their structural determination to find the nature of active bioactive compound that may be useful for clinical studies for the pharmaceutical industry to develop a new antibacterial, and anti-mycobacterial drug in future.

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Conflict of Interest

Authors have no conflict of interest.

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