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Collection, molecular identification and Antibacterial activity of *Acanthurus mata* marine fish from Visakhapatnam coast

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Abstract
A surgeonfish was collected from Visakhapatnam coast and molecularly it was identified as *Acanthurus mata.* An initial attempt on the antibacterial activity of the surgeonfish (*Acanthurus mata*) against the few human pathogenic bacteria. Two gram positive and two-gram negative bacteria were used as test organisms in the present study. Bacterial inhibitions were estimated by the cupplate methods and Minimal Inhibition Concentrations (MIC) were also estimated in the study. In the present study skin protein showed 15 mm zone of inhibition at 10mg concentration against *S. aureus* and Ovaries protein and lipid extracts showed maximum 16mm and 15 mm zone of inhibitions against *V. cholera.* Lower MIC values (320 µg/ml) was found for Skin protein extract in case of *S. aureus,* and *Salmonella* organisms on the other hand same value was found for the ovaries protein extract against *V. cholerae.*

Keywords: *Acanthurus mata*, antimicrobial, barcoding and MIC

1. Introduction
The biological organisms available in the marine ecosystem have novel and potent biomolecules. They may be includes many natural organic compounds. These compounds are reported to have biological activities like antimicrobial, anti-inflammatory, anti- tumor, anti- viral and analgesic (Rajamanikandan *et al*., 2011) [11]. The activities because of bioactive compounds or peptides or lipids (Khora, 2013) [7]. So many studies were intended to find antimicrobial compounds in the present context of increasing need for novel drugs that can control new illness or resistant strains of microorganisms (Williams *et al*., 2007) [13].
Acanthurus mata is a marine tropical fish belonging to the family of Acanthuridae or surgeon fishes. Its common names are elongate surgeonfish and blue-lined surgeon fish. *Acanthurus mata* has a diurnal activity. It is solitary when resting on the reef but may form small aggregation in the open water during feeding. It is found from the Red Sea and Gulf of Oman, south to Natal, eastwards to Society and Marquesan Islands, northwards to southern Japan, southwards to New South Wales and New Caledonia. Juveniles occur as far south as Sydney. It is reported from Western Australia (Allen and Swainston, 1988) [1], south to Shark Bay. It is not known to occur from the Hawaiian Islands, Pitcairn Islands and Rapa (Randall, 2001) [12]. In the present study skin, liver and ovaries of *Acanthurus mata* extracts were evaluated for their antimicrobial activities.

2. Materials and Methods
2.1 Sample Collection
Healthy marine fishes *Acanthurus mata* was collected during May 2016 from Tenneti beach (Long: 83º20' 59.94" E; Lat: 17º 44' 48.36" N) have been situated on the North East coast of Andhra pradesh, adjoining the Bay of Bengal, Visakhapatnam, India (Figure 1). Using hand nets, trammel nets, cast nets. The collected samples were aseptically placed in ice filled containers and transported to the laboratory. First sample washed under tap water and then with distilled water. Each sample was filleted - muscle tissue was separated from the skin with a knife. Three replicate samples were prepared. The dissected samples were stored at -20ºC for further analysis.

2.2 DNA extraction from fish muscle
Fish muscle tissue was suspended in 500 µl of buffer (20 mM Tris-Cl, 5 mM EDTA, 400 mM NaCl, 1% (w/v) SDS, 400 µg/ml Proteinase K), homogenized overnight at 55ºC in a shaking incubator with oscillation of 200 rpm. Equal volume of Phenol: Chlorophorm:Isoamyl Alcohol (25:24:1) was added to the dissolved liquid, placed in shaking incubator at room temperature for 30 min and centrifuged at 13000 rpm for 5 min. Upper aqueous layer was transferred to a new microcentrifuge and equal volume of isopropanol was added and centrifuged again at 8000 rpm for 15 minutes. The isopropanol was removed and the remaining was washed with 70% ethanol. Air dried DNA was resuspended in nuclease free water.

2.3 DNA barcode library construction
The COI standard barcoding region (652bp) was amplified for two fish samples using a pair of degenerate fish primers (Fish-Uni-F 5'-CACGACGTTGTAAAACGACACYAAICAYAAAGAYATIGGCAC-3'; Fish-uni-R- 5'-GGATAACAATTTCACACAGGACITCAGGGTGWCCGAARAAYCARAA-3') as well as a primer cocktail previously described. Each amplification reaction contained 2µl DNA template, 17.5µl molecular biology grade water, 2.5µl 10X reaction buffer, 1µl MgCl2 (50µM), 0.5µl dNTPs mix (10 mM), 0.5µl forward primer (10µM), 0.5µl reverse primer (10µM), and 0.5µl Invitrogen's Platinum Taq polymerase (5U/µl) in a total volume of 25µl. The PCR conditions were initiated

with a heated lid at 95 °C for 5min, followed by a total of 35 cycles of 94 °C for 40 S, 51 °C for 1min, and 72 °C for 30 S, and a final extension at 72 °C for 5min, and hold at 4 °C. PCR reactions were carried out using Mastercycler ep gradient S (Eppendorf, Mississauga, ON, Canada) thermal cyclers. PCR success was verified by 1.5% agarose gel electrophoresis. A DNA template negative control reaction was included in all experiments to test for contamination. Two microliters of each amplicon were subsequently used directly for bi-directional Sanger sequencing using Applied Biosystems's BigDye Terminator chemistry V3.1. Identification of the tested samples was conducted using BLAST in GenBank and a local barcode library for selected taxa with a minimum BLAST cut off of 98% identity for a top match.

2.4 Extractions
Three fish parts (Skin, liver and ovary) were separated, washed and allow them for extraction in the below methods.

2.4.1 Methanolic extraction
Hundred gm of fish parts were weighed and transferred into conical flask. Then 1000ml methanol was added. The conical flask was closed by foil paper and put in dark place at maximum 7 days. The crude methanol extracts were then filtered by passing the extracts through filter paper. After filtration, the extracts were placed in rotary vacum evaporator to concentrate.

2.4.2 Protein extraction
Ferreira *et al.*, (2002) [6] method was used to extract the total protein by using poly vinyl pyrrolidone (PVP). 5 gm of skin and tissue samplesof *Acanthurus mata* and was homogenised separately in 50 mM sodium phosphate buffer containing 10% insoluble PVP and incubated at 40ºC for overnight. Then the homogenates were centrifuged at 14000 rpm for 20 min at 4ºC. The supernatant was collected and stored at -20ºC for further protein research analysis.

2.4.3 Lipid extraction
Bligh and Dyer, (1959) [2] method was used for the extraction of total lipids from the skin tissues of *Acanthurus mata*. 1 gm skin from each sample was homogenised separately with 3.75 ml methanol: chloroform (2:1V/V) and 1 ml of 1 mM EDTA in 0.15 Macetic acid was added. Homogenate was transferred to new glass tube and the homogenizer rinsed with 1.25 ml of chloroform and transferred to the tube, finally1.25 ml of 0.88% KCl was added and centrifuged at 3000 rpm for 2 min. The lower phase which contains lipid was transferred to new tube. The lipid was stored in a refrigerator at 4-6°C for further analysis.

2.5 Antibacterial activity
2.5.1 Microorganisms
The below said microorganisms were obtained from IMTECH Chandigarh. The organisms were, *Bacillus subtilis, Salmonella, S. aureus* and *V. cholera.*

2.5.2 Agar-well diffusion testing
Antibacterial activities of the compounds investigated were first evaluated by agar-well diffusion method. The standardized cultures of test bacteria were first evenly spread onto the surface of Mueller Hinton Agar plates using sterile cotton swabs. Five wells (6 mm diameter) were made in each plate with sterile cork borer. Fifty microliters of each of the compound and positive control was added in wells. Gentamicin (200 µg/mL), vancomycin (1 µg/mL) were used as reference antibiotics. Diffusion of compounds, antibiotics and DMSO were allowed at room temperature for 1 h. All of the plates were then covered with lids and incubated at 37 ºC for 24 h. After incubation, plates were observed for zone of bacterial growth inhibition. The size of inhibition zones was measured and antimicrobial activity of the compounds was expressed in terms of the average diameter of inhibition zone in millimeters. Those compounds that were unable to exhibit inhibition zone (inhibition zone diameter less than 6 mm) were considered non-active. Each compound was tested in triplicate with two independent experiments and mean values of inhibition zone diameters were taken.

2.5.3 Determination of minimum inhibitory concentration and minimum bactericidal concentration
The minimum inhibitory concentration (MIC) values of the plant extracts were determined by microbroth dilution method. The test bacteria from the stock cultures were inoculated in MHB and incubated at 37°C under stirring for 24 h. The bacterial suspensions were then diluted with fresh MHB to achieve the turbidity equivalent to 0.5 McFarland standard. Different dilutions to get the final concentration ranging from 49 to 25000 µg/mL for the plant extract and 1 to 256 µg/mL for the reference antibiotics (gentamicin and vancomycin) were prepared in MHB directly in the wells of 96-well plates in a final volume of 200 µL. In each of these dilutions, 100 µL was of bacterial suspension (approx. 1.5 x 10^6 CFU/mL). The highest percentage of DMSO being used in the wells was 2.5 % and was not found to inhibit the growth of test bacteria. The wells containing 100 µL MHB, 100 µL bacterial inoculum and DMSO at a final concentration of 2.5 % served as negative control. The plates were covered with sterile plate sealer and were agitated to mix the contents of the wells by using shaker. Then the plates were incubated at 37oC for 18 h. After incubation, 40 µL of 0.2 mg/ml p-iodonitrotetrazolium violet indicator solution was added to every well in order to assess the bacterial growth. This indicator solution changes its color from colorless to red in the presence of bacterial growth and the degree of redness is a good indicator of inhibitory effect of extract or antibiotic on bacterial growth. After addition of this indicator solution, the plate was incubated for an additional 30 minutes. The MIC was defined as the lowest concentration of extract or antibiotic in which there was no visible growth of a test bacterium.

3. Results and Discussion
Total 648 base pairs sequence was received; the sequence was subjected to NCBI Blast analysis to know the similar sequences. 99.85% similarity was found for the *Acanthurus mata* voucher 243.5 cytochrome oxidase subunit 1 (COI) gene. And in the UPGMA tree analysis also the query sequences was present in the same group with *Acanthurus mata* (Figure 2). And the physical characters also similar to that organism.

> *Acanthurus mata* TAT CTAGTATTCGGT GCTTG AGCTGGGATAGTAGGAACGGCTCTAAGCCTCCTAA TCCGAGCAGAATTAAGCCAACCAGGCGCCCTCCTA GGGGATGACCAGATTTATAATGTAATTGTTACAGC

ACATGCATTCGTAATAATTTTCTTTATAGTAATACC
AATTATGATTGGTGGGTTTGGAAACTGATTAATTCC
ACTAATGATCGGAGCTCCTGATATAGCATTCCCAC
GAATGAACAATATGAGCTTTTGACTACTACCGCCA
TCTTTCCTATTATTACTTGCATCCTCCGCAGTAGAA
TCCGGCGCCGGTACGGGATGAACAGTTTATCCTCC
TCTAGCCGGTAACCTTGCACATGCAGGAGCATCCG
TAGACTTGACTATTTTCTCCCTTCACCTCGCAGGAA
TTTCCTCAATTCTTGGGGCTATTAACTTTATTACAA
CAATCATTAATATAAAACCCCCTGCTACTTCTCAAT
ATCAAACCCCTTTATTTGTATGAGCAGTATTAATTA
CTGCCGTTCTACTACTCCTTTCACTTCCCGTTCTTGC
TGCTGGAATTACAATACTACTCACAGACCGAAACC
TAAATACCACCTTCTTTGACCCGGCAGGCGGAGGA
GATCCCATTCTATATCAACATTTA

3.1 Antibacterial activity of *Acanthrus mata*

We have done antibacterial activity using the three parts of the *Acanthrus mata* i.e. skin, liver and ovaries. From each part protein, lipid and methanolic compound extracts were separated and antibacterial activity was done for them.

In *Acanthrus mata* three samples studied were showed antibacterial activity against the tested four organisms. All the three samples showed similar activity. In the four bacteria studied *S. aureus* was more sensitive than the remaining organisms, it showed 15mm and 13 mm zone of inhibitions at 10mg and 5 mg of compound respectively. *Salmonella, Bacillus subtilis and V. Cholera* were in the next place of showing inhibition activity. In case of gram-positive micro-organisms consistently protein extract was found to be better antibacterial activity. In *B. subtilis* lipid of ovaries showed 11 mm of zone of inhibition and in case of *S. aureus,* skin protein showed 15 mm zone of inhibition at 10mg concentration. Two-gram native organisms were used for the study, among them skin protein showed maximum 14 mm zone of inhibition against *Salmonella* bacteria. Ovaries protein and lipid extracts showed maximum 16mm and 15 mm zone of inhibitions against *V. cholera* (Table - 1). Based on the complete results revealed that protein extract showed maximum bacterial inhibitions. Minimal Inhibition Concentration (MIC) values were varied with each extract. Lower MIC values (320 µg/ml) was found for Skin protein extract in case of *S. aureus,* and *Salmonella* organisms on the other hand same value was found for the ovaries protein extract against *V. cholerae* (Table - 2). Similar kind of results also reported by a puffer fish *A. hispidus* from mandapam coast (Mohana and Khora, 2013) [9] and other similar results from *A. immaculatus* from a Parangipettai coast (Kumaravel *et al*., 2011) [8] which reports antibacterial activity against various pathogens. These results were indicating that the biological toxins present in the fish were having potential pharmaceutical activities. These toxins were used in health treatment due to its original binding ability, yet it show potentials as a drug in lower doses.

In our study the antibacterial activity of the skin and other tissues extracts of the surgeon fish *Acanthurus mata* against bacterial pathogens is not much notable which can be suggested for the medical usage. These antibacterial activity results are not unforeseen because the mucus is the biological interface between the fish and the aqueous environment and is composed of biochemically diverse secretions (Pickering, 1974; Ellis, 2001) [10, 5] that creations plays an important role in inhibition of the parasitic colonization bacteria and fungi on the surface (Ebran *et al*., 2000; Caccamese *et al*., 1980) [4, 3]

Table 1: Zone of inhibition antibacterial activity of extracts of *Acanthrus mata*

		Zone of inhibitions in mm							
		Gram positive				**Gram negative**			
		Bacillus subtilis		***S. aureus***		***Salmonella***		***V. cholerae***	
		10 mg	5 mg	10 mg	5 mg	10 mg	5 mg	10 mg	5 mg
Skin	Protein	10	8	15	13	14	11	9	0
	Methanol	8	0	12	8	10	8	0	0
	Lipid	10	8	14	11	12	10	8	8
Liver	Protein	9	0	13	11	9	8	12	10
	Methanol	8	0	8	0	8	0	9	0
	Lipid	9	7	12	10	7	0	12	9
Ovaries	Protein	10	8	8	7	8	7	16	10
	Lipid	11	9	8	9	6	0	15	10
	Methanol	0	0	7	0	7	0	0	0
Gentamicin (200 µg/mL)		21		19		17		19	

Table 2: Minimal Inhibition Concentration values of *Acanthrus mata* extracts

		MIC (µg/ml)			
		Bacillus subtilis	***S. aureus***	***Salmonella***	***V. cholerae***
Skin	Protein	620	320	320	725
	Methanol	640	430	560	0
	Lipid	620	340	355	820
Liver	Protein	650	355	620	560
	Methanol	725	650	655	655
	Lipid	650	560	725	560
Ovaries	Protein	620	725	655	320
	Lipid	560	725	1400	355
	Methanol	0	890	725	0

Fig 1: Marine collected sample of *Acanthrus mata*

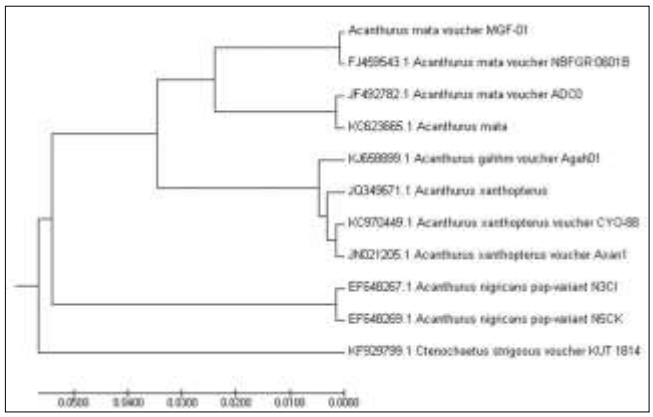

Fig 2: Evolutionary relationships of taxa using UPGMA method

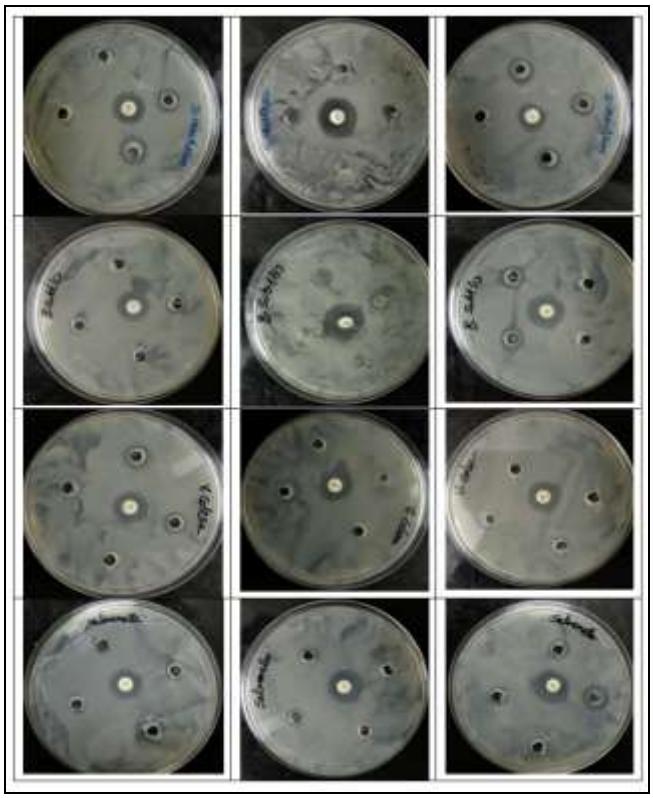

Fig 3: Anti-bacterial plates showing zone of clearance with *Acanthurus mata* extracts against salmonella. First row liver extracts, second row ovary extracts and third row skin extracts.

4. Conclusion

The collected fish in the Visakhapatnam coast was molecularly confirmed as *Acanthurus mata* by doing the sequencing BLAST of COI region in mitochondrial gene. We have studied antibacterial activity of the different parts (Liver, Skin and ovaries) of the fish and in the form of protein extract, lipid extract and methanolic extracts. Among the all skin protein produced higher antibacterial activity and it produced very less (320 µg/ml) MIC value.

5. References

1. Allen GR, Swainston R. The Marine Fishes of North-Western Australia. Western Australian Museum, Perth, Australia, 1988, 201pp
2. Bligh EG, Dyer WJ. A Rapid method of total lipid extraction andpurification. Can. J Biochem. Physiol, 1959; 37:911-917.
3. Caccamese S, Azzolina G, Furnari M, *et al*. Antimicrobial and antiviral activities of extract from Mediterranean algae. Bot Mar, 1980: 23:505-507.
4. Ebran N, Julien S, Orange N, *et al*. Isolation and characterization of novels glycoprotein's from fish epidermal mucus: correlation between their pore forming properties and their antibacterial activities. Biochim Biophys Acta. 2000; 1467(2):271-280
5. Ellis A. Innate host defence mechanisms of fish against viruses and bacteria. Dev Comp Immunol. 2001; 25(8):827-839.
6. Ferreira RR, Fornazier RF, Vitoria AP, Lea PJ, Azevedo RA. Changes in antioxidant enzyme activities in soybean under cadmium Stress. J Plant Nutr. 2002; 25(2):327-342.
7. Khora SS. Marine Fish-Derived Bioactive Peptides and Proteins for Human Therapeutics. Int J Pharm Pharm Sci. 2013; 5(3):31-37.
8. Kumaravel K, Ravichandran S, Sharmila J, Manikodi D, Mauro D. *In vitro* Antimicrobial Activity of Tissue Extracts of Puffer fish Arothron immaculatus Against Clinical Pathogens. Chinese J Nat Medicines. 2011; 9(6):446-449.
9. Mohana PK, Khora SS. Antimicrobial, Hemolytic and Cytotoxic activities of the Puffer Fish Arothron hispidus from the Southeast Coast of India. Int J Drug Dev Research. 2013; 5(2):317-322.
10. Pickering A. The distribution of mucous cells in the epidermis of the brown trout Salmona truta and the char Salvelinus alpines. J Fish Biol. 1974; 6(2):111-118.
11. Rajamanikandan S, Sindhu T, Durgapriya D, Anitha JR, Akila S, Gopalakrishnan VK, *et al*. Molecular docking and QSAR studies on bioactive compounds isolated from marine organisms into the MUC1 Oncoprotein. Int J Pharm Pharm Sci, 2011; 3:168-172.
12. Randall JE. Surgeonfishes of Hawai'i and the world. Mutual Publishing and Bishop Museum Press, Hawai'I, 2001, 123 p
13. Williams GP, Babu S, Ravikumar S, *et al*. Antimicrobial activity of tissue and associated bacteria from benthic sea anemone Stichodactyla haddoni against microbial pathogens. J Env Biol. 2007; 28(4):789-793.