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Original Article

Antimicrobial activity of striped eel cat fish *Plotosus lineatus* against human pathogens

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ABSTRACT

In this study striped eel cat fish were screened especially for their bioactivity, and the tests showed reproducible activity in the assay. Different parts like skin and spine of striped eel cat fishes were extracted with five different solvents. The crude extracts were tested against seven bacterial and five fungal pathogens. Maximum antibacterial inhibition zone was exhibited from n-butanol crude extract of Skin against *Klebsiella pneumoniae* 22 mm. In antifungal assay the chloroform extracts of skin showed the best activity against *Aspergillus fumigatus* of 16mm. This research is a baseline study for the development of the novel antimicrobial drug from fish.

Key words: Antibacterial, antifungal, pathogens

INTRODUCTION

The marine environment, which contains a vast array of organisms with unique biological properties, is one of the most underutilized biological resources. To date, algae and microalgae are referenced in the literature as sources of bioactive compounds for use as functional food ingredients [1, 2]. Bioactive compounds were isolated previously from other marine organisms including crustaceans, fish, and there by-products. In spite of modern improvements in chemotherapeutic techniques, infectious diseases are still an increasingly important public health issue [3].

Therefore, still a need for new methods of reducing or eliminating pathogens, possibly in combination with existing methods [4]. To combat this, several investigations have been focused to find out effective methods to prevent or cure diseases. Now-a-days the development of resistance by a pathogen to many of the commonly used antibiotics provides an impetus for further attempts to search for new antimicrobial agents to combat infections and overcome problems of resistance and side effects of the currently available antimicrobial agents. Identifying a novel antimicrobial compounds which is inherent to specific organ and tissues of an organism could be the potential alternative to combat the drug resistant bacteria pathogens. In this regard several studies have been made to explore the new antimicrobial drugs from natural sources including plant and animal origins. Antibacterial activity in fish mucus has been demonstrated in several fish species [5, 6, and 7]. Mucus thus acts as an immediate defense barrier to invasion and or colonization of pathogens [8]. In fish, the specific immune mechanisms are slow and limited by temperature constrains on their metabolism. Marine fishes are able to produce bioactive compounds on epidermal mucus that contain antibacterial activity to protect them from dangerous pathogens. Antibacterial activity was detected on mucus samples of salmon and cod. The striped eel cat fish, *Plotosus lineatus* belonging to plotosidae family which was found abundantly in kanyakumari rocky tidal areas and it contains toxin. Therefore the present study was conducted to extract potential bioactive compounds from eel cat fish.

MATERIALS AND METHODS

Sample collection and extract preparation: Live fish samples were collected from the Kanyakumari coastal waters and were immediately brought to the laboratory in an aerated container. Mucus sample was collected by swabbing method, using sterile swabs. The animals were washed thoroughly with fresh water to remove the debris, salt and air dried. The whole body of the samples were cut in to small

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pieces and air-dried for 24 hours at room temperature before extraction with solvents. Then the cut parts like skin and spine were rinsed with sterile distilled water and were used for extraction in different solvents, such as n-Butanol, Methanol, Chloroform, Acetone and Ethanol. The extracts were cold steeped overnight at -18^{0}C, filtered with Whatman No.1 filter paper. The filtrate was poured in previously weighed petri plate, evaporated to dryness in rotary evaporator and the dried crude extracts were used for antibacterial assay against both human and fungal pathogens. The antibacterial activity of the crude extracts was assayed by the standard Nathan's Agar well Diffusion (NAWD) technique against 7 human pathogens such as *Bacillus subtilis*, *Vibrio cholerae*, *Staphylococcus aureus*, *Streptococus pneumoniae*, *Escherichia coli*, *Salmonella typhi* and *Klebsiella pneumoniae* (CMC Vellore) and five fungal pathogens like *Aspergillus fumigatus*, *Candida glabrata*, *Candida albicans*, *Rhizomucor meihei* and *Candida tropicalis* on Muller Hinton Agar and Potato Dextrose Agar in Petri dishes with drilled wells of 6 mm diameter, crude dried extract was loaded on to each wells. The wells at the centre served as control (without the extract). After 22-24 hours of incubation at room temperature, the susceptibility of the test strain was determined by measuring the radius of zone of inhibition around each well which is the distance between the border of the well and the edge to which the test strains are completely inhibited.

RESULTS

Antibacterial activity: The antimicrobial activity of different extracts of spine and skin of striped eel cat fish, *Plotosus lineatus* were assessed both against bacterial and fungal pathogens using different solvents like acetone, methanol, n-butanol, ethanol and chloroform. Ethanol skin extracts exhibited an antibacterial activity of 13mm against *Bacillus subtilis* and minimal activity of 3mm against *Vibrio cholera*, methanol spine extracts showed a maximum activity of 10mm against *Bacillus subtilis*, and minimum 2mm against *Vibrio cholera.* The methanol skin extracts was able to inhibit of and *Klebsiella pneumoniae* with a maximum zone of 12mm and minimum 2 mm against *Streptococcus pneumoniae* the spine extract showed maximum activity against *Bacillus subtilis* 10mm and minimum 2mm against *Streptococcus pneumoniae.* Butanol skin extracts exhibited a spectral activity of 22mm against *Klebsiella pneumoniae* and minimal activity of 3mm against *Salmonella typhi*. Broad spectral activity was exhibited by n-butanol extracts of spine with about 11mm against *Klebsiella pneumoniae* minimal activity of 3mm against *Salmonella typhi.* Acetone skin extracts exhibited a broad spectral activity of 19mm against *Klebsiella pneumoniae* and minimal activity of 2mm against *Vibrio cholera.* Antibacterial activity of acetone extracts of spine showed maximum activity of 18mm against *Bacillus subtilis*, and minimal activity 3mm against *Vibrio cholera.* Chloroform skin extracts exhibited a broad spectral activity of 20mm against *Klebsiella pneumoniae* and minimal activity of 2mm against *Staphylococcus aureus a*nd chloroform extracts of spine with a effect of14mm against *Escherichia coli* and 2mm against *Streptococcus pneumoniae* (Table.1)

Antifungal fungal: Ethanol skin extracts exhibited an antifungal activity of 15mm against *Candida glabrata* and minimal activity of 5mm against *Aspergillus fumigatus*, methanol spine extracts showed a maximum activity of 10mm against *Candida albicans*, and minimum 4mm against *Candida glabrata.* The methanol skin extracts activity against C*andida albicans* with a maximum zone of 9mm and minimum 3 mm against *Candida glabrata* the spine extract showed maximum activity against *Candida albicans* 10mm and minimum 2mm against *Candida glabrata.* Butanol skin extracts exhibited a spectral activity of 12mm against *Candida glabrata* and minimal activity of 7mm against *Candida tropicalis*. Broad spectral activity was exhibited by n-butanol extracts of spine with about 9mm against *Candida albicans* minimal activity of 2mm against *Aspergillus fumigatus.* Acetone skin extracts exhibited a broad spectral activity of 15mm against *Candida albicans* minimal activity of 2mm against *Aspergillus fumigatus.* The activity of acetone extracts of spine showed maximum activity of 10mm against *Candida albicans* minimal activity of 2mm against *Candida glabrata.* Chloroform skin extract showed broad spectral activity of 16mm against *Aspergillus fumigatus* and minimal activity of 2mm against *Candida albicans.* Chloroform extract of spine showed the maximum inhibitory activity of 13mm against *Candida tropicalis*, and minimum 3mm against *Rhizomucor meihei* (Table.2)

DISCUSSION

The antibacterial and antifungal activities were prominated in the crude extract of *Plotosus lineatus.* In the present study, methanol skin extracts exhibited a broad spectral activity of 12mm against *Klebsiella pneumoniae* and minimal activity of 2mm against *Streptococcus pneumoniae* which coincides with the results of [9] who reported that the 100% methanol fraction of the egg masses of *Chicoreus virgineus* inhibited the fish pathogens, *Vibrio* sp, *Micrococcus* sp, more or less similar result was obtained against fungal

pathogens with an activity of 9mm against *Candida tropicalis.* The hypobranchial glands of *Chicoreus virgineus* and egg capsules of *Rapana rapiformis* extracted with polar solvents like ethanol and methanol also have been reported to show wide spectral antibacterial activities [10]. In this study the acetone extracts of spine showed activity of about 18mm against *Bacillus subtilis* and 10mm against *Candida albicans,* acetone skin extracts exhibited a broad spectral activity of 19mm against *Klebsiella pneumoniae* and 15mm against *Candida albicans.* The crude extraction from *Pteria chinensis* were used 6 solvents, the extract obtained from acetone exhibited higher antibacterial activity against human and fish pathogens [11]. Butanol body extracts of *Stichodactyla haddoni* tested against human pathogens, the pathogen *K. pneumonia* (11mm) and fish pathogen *Aeromonas hydrophila* shows higher activity of (22mm) [12] similar results were observed in this study. Chloroform skin extracts exhibited a broad spectral activity of 20mm against *Klebsiella* and 16mm against *Aspergillus fumigatus* and minimal activity of 2mm against *Candida albicans* were also shown by the chloroform skin extract. These findings are supported by [13] who stated that lipophilic chloroform extracts from the molluskan egg masses showed more antibacterial activity than polar extracts. The ethanol skin extracts was able to inhibit *Bacillus subtilis* with a maximum zone of 13mm, the fungal pathogen *Candida glabrata* was inhibited with a zone of 15mm. Antibacterial activity of epidermal layer of fin fishes was carried out against human pathogens by [14] and found that the crude methanol extract was good enough to bring the antibacterial activity against all the tested human pathogens. Also, there is a finding to report that the acetone extracts of different seaweeds showed antibacterial properties against human pathogens [15]. Use of fish skin or mucus for biologically active compounds are in research today most of these antimicrobial macromolecules have been isolated from fish skin that constitutes a first line barrier against microbial invasion. The mucus layer covering the integument of fish has mechanical protective functions.

CONCLUSIONS

In the present study revealed the striped eel catfish *Plotosus lineatus* spine and skin have higher potential to produce broad spectral antibacterial compound against a wide range of human and fungal pathogens. Further purification of the active compounds is necessary in order to clinically evaluate the potential of the newly derived compounds as novel drugs. The structural characterization of these molecules promote the utilization of this active principle as a lead in case of drug discovery from animal based formulations to control the emerging infectious drug resistant pathogenic microorganisms.

Table 1. Antibacterial activity of striped eel catfish against bacterial pathogens

mm (mili meter)

Organisms	Zone of inhibition (mm)									
	Ethanol		Methanol		Butanol		Acetone		Chloroform	
	Skin	Spine	Skin	Spine	Skin	Spine	Skin	Spine	Skin	spine
Vibrio cholerae	3	2	5	5	4	10	2	3	5	3
Salmonella typhi	3	3	4	3	3	3	4	4	3	2
Klebsiella pneumoniae	10	4	12	8	22	11	19	13	20	13
Escherichia coli	3	3	4	5	4	8	3	10	4	14
Bacillus subtilis	13	10	8	10	5	9	5	18	3	2
Streptococcus pneumoniae	13	3	2	2	3	4	6	6	2	13
Staphylococcus aureus	3	6	2.5	8	5	5	3	3	2	2

Table 2. Antifungal activity of striped eel catfish against fungal pathogens

Organisms	Zone of inhibition (mm)									
	Ethanol		Methanol		Butanol		Acetone		Chloroform	
	Skin	Spine	Skin	Spine	Skin	Spine	Skine	Spine	Skin	spine
A.fumigatus	5	8	4	8	10	2	2	3	16	3
Candida .tropicalis	12	10	9	5	7	5	2	5	2	13
Candida.glabarata	15	4	3	2	12	3	3	2	2	5
Candida.albicans	9	10	5	10	8	9	15	10	12	3
Rhizomucor meihei	8	8	7	7	9	5	2	7	3	2

mm (mili meter)

REFERENCES

1. Plaza M et al. Innovative natural functional ingredients from Microalgae. Journal of Agricultural and Food Chemistry 2009; 57: 7159-7170.
2. Plaza M et al. In the search of new functional and food ingredients from algae. Trends in foods science and technology 2008; 19: 31-39.
3. WHO. Food safety and foodborne illness. World Health Organisation Fact sheet 2002; 237. Geneva.
4. Leistner. Hurdle effect and energy saving. In W.K. Downey, (ed) Food Quality and Nutrition. Applied Science Publ.London1978; PP.553.
5. Palaksha K J et al. Evaluation of non-specific immune components from the skin mucus of olive flounder (*Paralichthys olivaceus*). Fish Shellfish Immunol 2008; 24: 479–488.
6. Kitani Y et al. Antibacterial action of L amino acid oxidase from the skin mucus of rockfish *Sebastes schlegelii*. Comp Biochem Physiol B 2008; 149: 394-400.
7. Raida M K, Buchmann K. Innate immune response in rainbow trout (*Oncorhynchus myiss*) against primary and secondary infections with *Yersina ruckeri*. Dev Comp Immunol 2009; 33:35-45.
8. Alexander J B, Ingram G A.. Noncellular nonspecific defence mechanisms of fish. Ann. Rev. Fish Dis 1992; 2, 249–279.
9. Sunahara S et al. Amino acid sequence of two sea anemone toxins from *Anthopleura fuscoviridis*. Toxicon 1987; 25(2): 211-219.
10. Ramasamy S M, Murugan A. Antifouling property of and possible chemical defense strategies in the star fish *Pentaceraster affinis* from Tuticorin, South east coast of India, proceedings of the National Seminar on Biodiversity as a source of food and Medicine, SDMRI Research Publication 2003; 35:129-132.
11. Anand T P. Antibacterial activity of marine molluscs from Porto Novo region. Indian. J. Mar. Sci 1997; 26: 206-208.
12. Chellaram C. Studies on the bioactive potential of coral reef associated bacteria and Molluscs of Tuticorin coastal waters, Southeastern India. Ph.D thesis. Manonmaniam Sundaranar University, Tirunelveli 2004.
13. Prakash Williams G et al. Antimicrobial activity of tissue and associated bacteria from benthic sea anemone *Stichodactyla haddoni* against microbial pathogens. J Environ Biol 2007; 28(4): 789-793.
14. Benkendorff K A et al. Chemical defence in the egg masses of benthic invertebrates: an assessment of antibacterial activity in 39 mollusks and 4 polychaetes. J. Invertebr. Pathol 2001; 78: 109-118.
15. Shani T J, Jamila P. Antibacterial activity of epidermal layer of fishes against human pathogens. Indian J. Fish 2011; 58(4):87-90.
16. Sureshkumar et al. Antimicrobial activity of acetone extracts of seaweeds against human pathogens. Seaweed Research Utilization 2002; 24 (1): 111-116