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Short Communication

Evaluation of anti-bacterial potential of protein isolated from the muscle of *Channa striatus*

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ABSTRACT:

Protein was extracted from muscles of *Channa striatus* and attempts were made to evaluate *in vitro* antibacterial activity against clinical bacterial isolates. The higher concentration of protein (100µg/ml) extracts exhibited a pronounced activity against *Pseudomonas aeruginosa* (21 mm), *Proteus vulgaris* (19 mm), *Citrobacter* sp (19 mm), *Klebsiella pneumoniae* (18 mm), *Micrococcus* sp (17 mm), *Bacillus subtilis* (16 mm), *Staphylococcus aureus* (15 mm), *E. coli* (14 mm) and *Serratia marcescens* (5 mm). The minimum inhibitory concentration and minimum bactericidal concentration were found to be 20-40 µg/ml and 80-100 µg/ml respectively for the extracts of *Channa striatus* protein against test organisms. This study confirms that *C. striatus* fish protein extracts possess antibacterial activity against a wide range of microbes and justified that it could be used in the traditional medicine as a remedy for the treatment of bacterial diseases.

Keywords:
Channa striatus, Anti-bacterial activity, MIC and MBC.

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INTRODUCTION

Fish being the most antiquated type of aquatic life as a food have been accounted to have a twofold nutritional benefit of having the capacity to give high extents of their quality because of the occurrence of essential amino acids. Furthermore being easily digestible not like those of meat and other domesticated animals as a result of low collagen contamination of natural habitat from modern, household and rural waste has uncovered these vital aquatic life forms to contaminants which endanger their lives as well as in the end enter the evolved way of life prompting serious health hazards (Ilavazhahan *et al.*, 2010).

Bacterial diseases of extraordinary artifact, has caused more suffering and death than most pathogens. It remains a noteworthy medical issue of worldwide concern (Grange, 1998). The current evaluations propose that around two billion individuals are infected with bacterial pathogens, of which 8-10 million create dynamic diseases with two million deaths every year (Frieden *et al.*, 2003). Barbosa and Levy (2000) revealed the extensive use and abuse of anti-biotic are without a doubt the real foroces related with the high quantities of resistant pathogenic and commensal microbes around the world. For diagnostic purposes pure protein derivate is used to distinguish immune response to the bacterial antigens (Sujatha *et al*., 2010). The advancement of antimicrobial medication protection is a noteworthy hindrance to infectious disease control. Strategies for disease prevention, for example, enhanced cleanliness, anti-microbials and vaccine developments have demonstrated to be fruitful in controlling numerous illnesses, however the adequacy of immunizations relies upon the standing and developing viability of serotypes in a given populace of bacterial pathogens. Future disease control depends on the vaccine development which is often constrained by failure to initiate sufficient resistant reactions to the full scope of disease causing serotypes of a specific pathogenic species seen in an environment (Tekle *et al.,* 2012). Subsequently, there is a need to distinguish and assess bacterial antigens for the diagnosis of bacterial disease and to create powerful and more secure vaccines for replacing allopathic medications. There are numerous diseases known today for which no strong vacccines exist, and the utilization of low efficacy immunizations may really diminish profitability (Thorarinsson and Powell, 2006).

Therefore, the present study was designed to study the medicinal use of proteins isolated from *Channa striatus* to explain the rationale of its use in traditional medicine by *in vitro* assessment. The antibacterial activities of protein extracts against clinical bacterial isolates of human pathogenic microorganisms were studied.

MATERIALS AND METHODS:

One gram of muscle sample was taken from *Channa striatus* (Murrel fish) collected from Poondi dam reservoir, Thiruvallur district, Tamilnadu, India. It was homogenized with distilled water using mortar and pestle for the preparation of crude sample. From the crude protein sample, proteins were isolated by ion exchange chromatography method (Zhang *et al.,* 2006). Before starting the separation, an equilibration buffer was pumped through the column to equilibrate the oppositely charged ions. Upon injection of the crude protein sample, solute particles were exchanged with the buffer ions as every ion would compete for the binary sites on the resin. The most weekly charged compounds were eluted in the first place, trailed by those with progressively stronger charges utilizing elution buffer. Purity of the eluted protein sample was affirmed by Western blot techniques (Paulsi and Dhasarathan, 2011).

Anti bacterial testing

Microorganisms employed determining the anti-bacterial activity of the extract are *Escherichia coli, Pseudomonas aeurogenosa, Bacillus subtilis, Proteus vulgaris, Klebsiella pneumoniae*, *Micrococcus*

Table 1. Antibacterial effect of the *Channa striatus* protein against pathogenic bacteria

S. No	Bacterial Culture	Zone of inhibition (cm) at different concentration of murel fish protein (µg/ml)					Streptomycin (µg/ml)	
		20	40	60	80	100	20	40
1	*Escherichia coli*	0.7	0.8	0.9	1.3	1.4	1.2	1.8
2	*Pseudomonas aeruginosa*	0.4	1.2	1.7	1.9	2.1	1.7	2.6
3	*Proteus vulgaris*	0.6	0.8	1.1	1.6	1.9	1.5	2.3
4	*Klebsiella pneumoniae*	0.4	0.5	1.5	1.6	1.8	1.6	2.2
5	*Citrobacter sp*	0.4	0.6	1`.5	1.6	1.9	1.5	2.2
6	*Serratia marcescens*	0.2	0.1	0.2	0.1	0.5	0.5	1.0
7	*Micrococcus sp*	0.2	0.8	1.2	1.5	1.7	1.4	2.0
8	*Staphylococcus aureus*	0.3	0.7	1	1.3	1.5	1.2	2.0
9	*Bacillus subtilis*	0.6	0.9	1.2	1.5	1.6	1.4	2.1

sp, *Staphylococcus aureus, Citrobacter* sp and *Seratia marcescens* which were isolated from the different patients diagnosed with various wound infections at the laboratory of Joys Clinical Lab, Manvalanagar, Thiruvallur, Tamilnadu. The antibacterial activities of *C. striatus* fish protein extracts were evaluated *in vitro* by disc diffusion method using Muller Hinton medium.

Disc diffusion method

Filter paper disc diffusion method (Garg and Jain, 1998) was used for analyzing antimicrobial activity. Whatman No.1 filter paper discs of 6mm diameter were, set in dry petri plates, and autoclaved. Sterile filter paper No.1 discs were loaded with the test protein extracts following Irobi *et al.* (1996) method. Discs were loaded with standard antibiotic streptomycin (w/v) in two various concentrations (20µg/ml and 40µg/ml). The pathogenic strains were suspended in Muller Hinton broth (Hi Media) by transferring a loop full of bacteria grown for 24 h on agar slants. The suspensions were vortexed and 0.1ml aliquots were spread over respective agar medium plates. The protein extracts and streptomycin loaded discs were then kept over the plates seeded with particular microorganisms. The plates were incubated at 37ºC for 24 h. The antibacterial action was determined by measuring the inhibition zone around the discs.

MIC and MBC test

Minimum Inhibition Concentration (MIC) of the extracts was resolved from the culture plate that had the most lowest concentration inhibiting the development of bacterial strains. Minimum Bacterial Concentration (MBC) was determined by utilizing the strategy for Samy and Ignacimuthu (2001). The test containing 3ml of Muller Hinton broth and 0.1ml of bacterial suspension and 0.1ml protein extract were incubated at 37ºC for 24 h. Bacterial turbidity was measured at 650 nm to decide the rate of inhibition of bacterial growth. Streptomycin at 20 and 40µg/ml was utilized as reference for detecting MBC. The tubes containing just the growth medium and each of the bacteria were utilized as control. The MBC indicated decrease the bacterial growth as measured from the turbidity of the culture examined by optical density value.

RESULT AND DISCUSSION

The proteins was characterized by Western blot method. Bands in test lane was compared with standard protein marker, which shows 136 kDa molecular weight. The protein extract of *C. striatus* collected from the Poondi dam reservoir was found to be effective against all tested organisms with inhibition zone ranging from 5 to 21 mm in the concentration of 100µg/ml. When, comparing the result with standard anti-biotic streptomycin a direct proficiency was recorded (Table 1). The protein extract of *C. striatus* collected from the Poondi dam reservoir showed highest inhibition activity

Table 2. The minimum bactericidal concentration of *C. striatus* protein against pathogenic bacteria

S. No	Concentration of extracts (µg/ml)	Minimum bactericidal concentration (optical density value) of protein isolated from *Channa striatus* of Poondi								
		E. coli	*P. aeruginosa*	*P. vulgaris*	*K. pneumoniae*	*Citrobacter sp*	*S. marcescens*	*Micrococcus sp*	*Staphylococcus aureus*	*Bacillus subtilis*
1	Normal	1.8	1.7	1.9	2.0	2.0	2.0	1.9	1.8	1.9
2	20	1.18	1.46	1.28	1.32	1.42	1.50	1.54	1.62	1.61
3	40	1.16	1.2	1.2	1.2	1.12	1.25	1.5	1.46	1.52
4	60	1.0	1.0	1.0	1.0	1.1	0.8	0.9	0.9	0.46
5	80	0.45	0.56	0.65	0.5	0.56	0.5	0.6	0.72	0.75
6	100	0.18	0.46	0.28	0.32	0.42	0.50	0.54	0.62	0.61
7	Streptomycin – 20	0.34	0.35	0.4	0.42	0.16	0.53	0.52	0.42	0.35
8	Streptomycin – 40	0.16	0.1	0.12	0.13	0.4	0.5	0.25	0.13	0.16

against *Pseudomonas aeruginosa* (21 mm) followed by *Proteus vulgaris* (19 mm), *Citrobacter* Sp (19 mm), *Klebsiella pneumoniae* (18 mm), *Micrococcus* sp (17 mm), *Bacillus subtilis* (16 mm), *Staphylococcus aureus* (15 mm), *E. coli* (14 mm) and *Serratia marcescens* (5 mm).

Channa striatus samples collected from Poondi dam reservoir had broad spectrum of antibacterial potential. The minimum inhibitory concentrations of fish protein, *C. striatus* was given in Table 2. The results showed that the minimum inhibitory concentration of fish protein of *C. striatus* showed the inhibitory effect against all test pathogens at the concentration of 20-40 µg/ml onwards.

The minimal bactericidal concentrations of the protein of *C. striatus* samples showed the notable effect on all test pathogens. The minimum bacterial concentration (80-100µg/ml) of the *C. striatus* fish proteins showed effect of pathogenic inhibition in comparison to those of streptomycin. *In vitro* antibacterial test was added to evaluate the viability of protein extract by inhibiting the growth of pathogenic organisms with wide range of antibacterial potential. Gram positive microorganisms and gram negative microbes like *Escherichia coli, Pseudomonas aeruginosa, Bacillus subtilis, Proteus vulgaris, Klebsiella pneumoniae, Micrococcus* Sp, *Staphylococcus aureus, Citrobacter* Sp and *Serratia marcescens* demonstrated a decrease in their development on treatment with the protein extract. The level of inhibition as measured by the disc diffusion method, announced that the gram negative bacteria were more hindered than the gram positive microbes. Similar findings were also reported by Lighty *et al.* (2010) in the extract of red velvet mites.

CONCLUSION

The present study suggested that using protein extracts of *C. striatus* fish is cheap and cost effective drugs can be prepared for bacterial infections. The broad spectrum of antibacterial activity of *C. striatus* fish protein is highly promising for further analysis. The active fraction obtained from this fish protein is an attractive material for further studies leading to possible drug development. This fraction can be used as such for ethno medicine development with further, studies to establish safety and efficacy. Improvement of ethno medicines is generally cheap and less tedious; it is more

Table 3. The MBC of *C. striatus* protein against pathogenic bacteria

S. No	Concentration of extracts (µg/ml)	MBC (optical density value) of protein isolated from *Channa striatus* of Poondi reservoir 1								
		E. coli	*P. aeruginosa*	*P. vulgaris*	*K. pneumoniae*	*Citrobacter sp*	*S. marcescens*	*Micrococcus sp*	*Staphylococcus aureus*	*Bacillus subtilis*
1	Normal	1.8	1.7	1.9	2.0	2.0	2.0	1.9	1.8	1.9
2	20	0.8	0.65	0.85	0.9	0.65	0.9	0.8	0.7	0.85
3	40	0.6	0.5	0.7	0.85	0.6	0.76	0.7	0.65	0.78
4	60	0.4	0.4	0.5	0.62	0.51	0.63	0.53	0.56	0.63
5	80	0.2	0.3	0.4	0.4	0.42	0.41	0.35	0.38	0.41
6	100	0.1	0.2	0.1	0.32	0.2	0.3	0.1	0.2	0.2
7	Streptomycin – 50	0.34	0.35	0.4	0.42	0.16	0.53	0.52	0.42	0.35
8	Streptomycin – 100	0.16	0.1	0.12	0.13	0.4	0.5	0.25	0.13	0.16

appropriate to our financial conditions contrasted with allopathic sort of drug advancement.

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