# Antibacterial activity of muscle extracts of mangrove red snapper from *Lujanus argentimaculatus* against enteric pathogens

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#### Abstract

Antibacterial activity of methanolic and acetone muscle extracts of *Lujanus argentimaculatus* against enteric pathogens was tested by standard disc diffusion method. Both the methanolic and acetone muscle extracts showed maximum zone of inhibition against *Staphylococcus aureus* and *Escherichia coli* at 30 µl concentration. It is suggested that the strong antibacterial effect might be due to the antimicrobial peptides present in it.

**Key Words:** Antibacterial activity, *Escherichia, Lujanus,* peptides, *Staphylococcus* 

#### INTRODUCTION

Since ea-rlier civilizations, fishing and seafood consumption have played a significant role in the coastal areas of Tamil Nadu. Seafood products seem to be a healthy diet worldwide. It is one of the good sources of animal protein, poly unsaturated fatty acids (Paramasivam et al., 2013) vitamins viz A, B12, D, E, and minerals like iodine, selenium, calcium, zinc and iron (Mashaii et al., 2012). In recent years, fish has become a favourite foodstuff for the majority of people owing to several health reasons. Fish diet plays a protective role against the development of cardiovascular diseases and rheumatoid arthritis (Kris-Etherton et al., 2003; Tedeschi et al., 2018). This pharmacological activity of fish diet is due to the macromolecules and its derivatives present in it. The mangrove red snapper Lujanus argentimaculatus is a large, predatory reef fish belonging to the family Lutjanidae. In Tamil it is called as Sankara. This fish is rich selenium, potassium and omega-3 fatty acids.

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It has been recognized as one of the most important aquaculture species.

Enteric or diarrhoeal infections are significant public health issues in developing countries and contribute to the death of 3.3 to 6.0 million children annually. Salmonella Shigella, Proteus, Klebsiella, Escherichia coli, Pseudomonas, Vibrio cholera and Staphylococcus aureus are the major etiologic agents of sporadic and epidemic diarrhea both in children and adults (Haniffa et al., 2013). Many diseases were initially controlled by the use of antimicrobial drugs. The over usage of existing antibiotics is a major threat to the medical field due to the emergence of multi drug resistant pathogens (Bandow et al., 2003). There is an urgent need to explore and discover new antimicrobial compounds with diverse chemical structures and novel mechanism of action against new infectious diseases. The rich diversity of marine ecosystem can be explored to discover new pharmacologically bioactive substances. This paper explores the antibacterial activities of muscle extracts of mangrove red snapper Lujanus argentimaculatus.

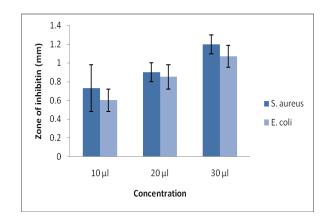
#### MATERIALS AND METHODS

#### Collection of animal specimen

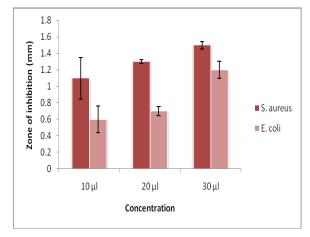
The fish purchased from the local fish market of Tiruchirappalli district, Tamil Nadu, India were brought to the laboratory and washed well until sand and mud were completely removed from it. The muscle was cut into small pieces rinsed with sterile distilled water, kept in petri dishes, dried at a constant temperature of 50 °C for 24 hrs in a hot air oven and powdered.

#### Preparation of muscle extract

Five gram of muscle powder was dissolved in 100 ml of methanol and acetone solvents at 18 °C to prepare 5% of methanolic and acetone muscle extract, respectively. The extracts from the solvent were filtered



**Fig. 1.** Antibacterial activity of methanolic extract of muscle of *Lujanus argentimaculatus* 



**Fig. 2.** Antibacterial activity of acetone extract of muscle of *Lujanus argentimaculatus* 

twice using Whatman No.1 filter paper and used for the experiments.

#### Antibacterial activity

#### **Bacterial cultures**

Gram positive bacteria *Staphylococcus aureus* (MTCC 3160) and Gram negative bacteria *Escherichia coli* (MTCC 443) were purchased from Microbial Type Culture Collection (MTCC), Chandrigarh, India. Each strain was sub cultured and maintained at 4<sup>o</sup> C and used to test antibacterial efficacy.

#### **Evaluation of Antibacterial activity**

Antibacterial activity of the methanolic and acetone muscle extracts were analysed by following the method of Sulaiman et al., (2013). The three different concentrations ( $10 \mu$ l,  $20 \mu$ l and  $30 \mu$ l) of 5% extract of the methanolic and acetone extract were tested for antibacterial activity using agar disc diffusion assay. Sterile discs of six millimeter width had been impregnated with different concentrations of samples

and introduced onto the upper layer of the seeded agar plate. The plates were incubated overnight at 37 °C. Antibacterial activity was assessed by measuring the inhibition zone formed around the discs in millimeter. The experiments were repeated in triplicates and the mean with S.D values were presented.

## Antibiotic sensitivity test on microbes (Positive control)

The antibiotic sensitivity test was analysed using standard antibiotics (Tetracycline for *S. aureus* and Erythromycin for *E. coli*). The sterilized nutrient agar medium was poured into each sterile petri plates and allowed to solidify. By using a sterile cotton swabs, a fresh bacterial culture with known population count was spread over the plates by following spread plate technique. Then the selected standard antibiotic discs were placed on the petri plates and were incubated for 24 hrs at 37° C. The results were observed and the diameter of the inhibition zone measured.

#### RESULTS

#### Antibacterial activity

The antibacterial activities of methanolic and acetone extracts muscle of *Lutjanus argentimaculatus* are shown in Figures 1 and 2. Both the extracts had efficient antibacterial activity against the pathogens (Figures 1 and 2). The zone of inhibitions ranged from 0.6 to 1.5 mm. Both the extracts showed maximum activity at 30  $\mu$ l concentration.

#### DISCUSSION

Among the fish by-products, fish mucus, gills and blood is considered more valuable and has been reported to contain several antimicrobial proteins (Kumar et al., 2012). Most of the antimicrobial peptides kill bacteria by a common mechanism, which involves direct electrostatic interactions with negatively charged phospholipids on microbial cell membranes followed by physical disruption and solubilization. Fish contain naturally occurring proteins and glycoproteins of non-immunoglobulin nature (transferrins, metallothionein) that react with environmental antigens and confer an undefined natural immunity to fish. Hence, several endogenous peptides with antimicrobial activity have been purified from fish especially from the skin and intestinal mucus.

In the present study, we have screened the antibacterial activity of mangrove red snapper fish *Lutjanus argentimaculatus* against two enteric bacterial strains with 5% methanolic and acetone extract of three different (10  $\mu$ l, 20  $\mu$ l and 30  $\mu$ l) concentrations. The antibacterial activity of methanolic muscle extract at 30  $\mu$ l concentration showed zone of inhibition as 1.20

mm and 1.07 mm against S. aureus and E. coli respectively. The acetone extract at 30 µl concentration showed maximum zone of inhibition as 1.51 mm against S. aureus and 1.36 mm against E. coli. Several antimicrobial studies have been done earlier using the mucus of fishes and their tissue extracts. Kumaravel et al., (2011) have shown maximum antimicrobial effect against S. aureus by liver extract and antibacterial effect against Vibrio cholera by skin extract of Puffer fish Arothron immaculatus. Five crude extracts from four Channa species showed maximum zone of inhibition as 12.6 mm for the methanol extract of C. striatus against Shigella dysenteriae (Haniffa et al., 2013). Mohana Priya et al., (2013) have screened the crude tissue extracts (Liver, Muscle, skin) of A. hispidus against seven human pathogenic bacteria for antibacterial activities. They observed maximum zone of inhibition 12.8 mm against the *E. coli* by the skin extract of *A.hispidus* and the minimum zone 9.60 mm against *P. vulgaris* by the liver extract. Anbuchezhian et al., (2011) have studied the spectrum of antibacterial activity of the mucus of estuarine cat fish A. maculatus and observed maximum (8 mm) against E.coli and minimum (6.5 mm) zone of inhibition against K. pneumonea.

#### CONCLUSION

The fish *L. argentimaculatus* can be used as supplements to patients especially those with protein deficiency. As per the nutritional status concerned it is a commercially sound species and further research should focus on the amino acids and fatty acids profile which will be beneficial to mankind. Also, the antibacterial efficacy of the tissue extract showed it can be used as natural antimicrobial agent replacing synthetic antibiotics.

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