INTRODUCTION:

Marine organisms have been attracting attention as potentially rich sources of structurally novel and biologically active metabolites with pharmaceutical interest (Kobayashi, 1989; Schmitz and Yagumoto, 1991; Stingl et al., 1992) The metabolites produced from marine organisms exhibit a variety of pharmacological activities viz. fungicidal, growth-inhibiting, antitumor, antiviral, antibiotics etc. Hence the search for novel and potential drugs from sea has been increasing not only in intensity but also in gaining prime importance (Baker and Murphy, 1976; Baslon, 1977).

Antibiotics substances are specific chemical compounds produced by living organisms, which inhibits the life processes of other organisms. Burkholder (1952) demonstrated the concept of special antagonistic substances elaborated by microorganisms growing in culture media and capable of inhibiting susceptible bacteria in the later part of the 19th century. Antimicrobial substances will be considered from the view point of the nature and origins of such substances and their importance as potential biomedical agents of significance to man. Until now the vast bulk of research in the field has been concerned with the screening of biological co-actions in suitable laboratory tests.

Different kinds of evidence may come from folklore, primitive medical practices, and microbiological screening and biochemical investigations. Marine biologists, chemists and pharmacologist will certainly continue to discover many diverse and important types of substances biosynthesized by fauna and flora of the sea. Of the new approved drugs reported between 1983 and 1994, drugs of natural origin predominate (78%) in the area of antibacterial (Cheng, 1995). This highlights the invaluable role that natural products have played and continue to play in the drug discovery process.
Today most infectious diseases can be brought under control with natural or synthetic drug products. There is still great need for safe, cost effective and more effective drugs. Some marine organisms have shown pronounced antibacterial activities, useful in the biomedical area. Antibacterial proteins are an important part of the innate immune system. They have received only scant attention in fish (Smith, et al., 2000). The potentiality of marine fishes as a source of biologically active products is largely unexplored. Previous studies on Pacific golden striped bass *Grammistes sexlineatus* produce a toxic substance active against *Escherichia coli* (Liguori et al., 1963). Renierone is a unique isoquinolinequinone, an antimicrobial metabolite from a blue marine sponge active against *Staphylococcus aureus* and *Bacillus subtilis* (Kuba and Nakahara, 1981).

The skin acid extracts of channel cat fish *Ictalurus punctatus* exhibited antimicrobial activity against Aeromonas (Robineette, 1998). Ebran et al., (2000) isolated a novel glycosylated protein from crude epidermal mucus extract of tench *Tinca tinca*, eel *Anguilla anguilla* and rainbow trout *Oncorhynchus mykiss* showed antibacterial activity against both gram -ve and gram +ve bacteria. The skin secretion of certain fish is possessed toxins causes ichthyotoxic, haemolytic activities presumably as defence substances against predators and microbial attack (Raghunath Ravi and Venkateswaran, 1999).

The crude methanol extract of mucus of *Narcine timlei* showed antibacterial activity against *Vibrio cholerae, Salmonella typhimurium, Shigella flexneri* (Yogamoorthi and Srikala, 2001). Even though reports on the antibacterial properties of marine fauna and flora are ample, the studies pertaining to entire fish are very meager.
Considering all the above facts, an attempt has been made to study the antibacterial properties of the crude methanol extracts of flat fish *Pseudorhombus elevatus* was examined against five common bacterial pathogens *viz.* *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus aureus* and *Vibrio cholerae*

**Materials and methods:**

Antibacterial activity of crude fish extracts was determined against five bacterial strains *viz.* *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus aureus* and *Vibrio cholerae*. The bacteria were obtained from Jawaharlal Institute of Post-Graduate Medical Education and Research, Puducherry. Nutrient broth medium was prepared and sterilized in an autoclave at 15 lb pressure. Five bacterial species were inoculated at about $10^8$ cells/ml. approximately in the nutrient broth and incubated at $28\pm2^\circ C$ for 24 hour.

In vitro antibacterial assays were conducted using the standard disc-diffusion technique (Mc Caffrey and Erdean, 1985). Known amount of extracts were impregnated to whatman No.1 paper discs with 6 mm diameter. Discs with standard antibiotics (paper disc loaded with Ampicillin, Chloramphenicol, Gentamycin and tetracycline) were also prepared and allowed to dry at room temperature. Further, they were aseptically placed on agar plates seeded with test microorganisms. The plates were incubated at $37\pm1^\circ C$ for 24 hr. After incubation, zones of growth inhibition around the paper disc were measured reverting the plates using an mm scale. All the experiments were carried out in triplicates at two concentration (50 and 100 μg/disc) and the average values were recorded.
RESULTS:

The antibacterial activity of the crude methanol extract of fish *Pseudorhombus elevatus* was measured as radius of zone of inhibition (diameter in mm) around the disc in comparison with the standards Ampicillin, Chloramphenicol, Gentamycin and Tetracycline.

The crude methanol extract of the fish *P.elevatus*, which showed significant inhibitory zone for the pathogenic bacteria *E.coli*, *K.pneumonia*, *S.aureus*, *S.typhi* and *V.cholerae*. When compared with standard drugs (dosage: 100μg/disc) used *viz* *Escherichia coli* (Ampicillin and Chloramphenicol), *Klebsiella pneumonia* (Gentamycin), *Staphylococcus aureus* (Chloramphenicol), *Salmonella typhi* (Ampicillin and Gentamycin) and *Vibrio cholerae* (Tetracycline).

The crude methanol extract of *P.elevatus* in the concentration of 50μg/disc and 100μg/disc expressed the inhibition zone of 12mm and 13mm respectively against the bacteria *E.coli*. In the case of the pathogenic bacteria *K.pneumonia*, the crude extract of methanol in the concentration of 50μg/disc and 100μg/disc was recorded maximum significant while comparing with the standard; the IZD was 9mm and 10mm respectively. The bacterial strain *V.cholerae* growth was suppressed by the crude methanol extract of *P.elevatus* in the concentration of 50 and 100μg/disc and measures the IZD 7mm and 8mm respectively. The crude methanol extract of *P.elevatus* exhibited inhibition zone of 11mm and 12mm and 11mm and 12mm against *S.aureus* and *S.typhi* in the concentration of 50 and 100μg/disc respectively.
DISCUSSION

The crude methanol extract of *Pseudorhombus elevatus* has moderate to strong antibacterial activity against *Escherichia coli, Klebsiella pneumoniae, Salmonella typhi, Staphylococcus aureus* and *Vibrio cholerae*. The inhibition of *E.coli* to the tune of 13mm IZD at 100µg/disc, which is comparable to the activity of standard antibiotic Chloramphenical (19mm IZD). The standard antibiotic like Ampicillin, Gentamycin, Chloramphenicol and Tetracycline used in the present study, belong to the group aminoglycoside antibiotics that inhibit the enzymatic polymerization of amino acids, responsible for the bacterial action (Warner and Warker, 1989). The important finding is the crude methanol extract of *P.elevatus* has moderate to strong antibacterial activity (7mm to 13mm) against almost all test pathogens. The methanol extract of *P.elevatus* exhibited a better activity at the concentration of 100µg/disc against *E.coli* (13mm IZD), *K.pneumonia* (10mm IZD), *S.aureus* (12mm IZD), *S.typhi* (12mm IZD) and *V.cholerae* (8mm IZD) was slightly less than the known antibiotic tetracycline activity (24mm IZD).

On the other hand inhibition exhibited by the crude methanol extract of *P.elevatus* against *S.aureus* (11mm IZD), *K.pneumonia* (9mm IZD), *E.coli* (12mm IZD), *S.typhi* (11mm IZD) and *V.cholerae* (7mm IZD) at the concentration of 50µg/disc was less than the standard antibiotics Gentamycin (19mm IZD) and Tetracycline (24mm IZD) also considered as slight significant.

Hence in the conclusion, the crude methanol extract of *P.elevatus* possess the bioactive property of inhibiting the growth of both gram +ve and gram –ve pathogenic bacteria, indicating its wide spectrum of antibacterial property.
P - 3a
Antibacterial activity of crude methanol extract of Pseudorhombus elevatus against Vibrio cholerae (Enlarged)
std - t: Tetracycline
1: 50 μg/disc
2: 100 μg/disc

P - 3b
Antibacterial activity of crude methanol extract of Pseudorhombus elevatus against Klebsella pneumonia std - g: Gentamycin
1: 50 μg/disc
2: 100 μg/disc

P - 3c
Antibacterial activity of crude methanol extract of Pseudorhombus elevatus against Klebsella pneumonia (Enlarged) std - g: Gentamycin
1: 50 μg/disc
2: 100 μg/disc
**P - 2a**
Antibacterial activity of crude methanol extract of Pseudorhombus elevatus against Staphylococcus aureus
*std - c ; Chloramphenical
1 ; 50 µg/disc
2 ; 100 µg/disc*

**P - 2b**
Antibacterial activity of crude methanol extract of Pseudorhombus elevatus against Escherichia coli
*std - c ; Chloramphenical
1 ; 50 µg/disc
2 ; 100 µg/disc*

**P - 2c**
Antibacterial activity of crude methanol extract of Pseudorhombus elevatus against Vibrio cholerae
*std - t ; Tetracycline
1 ; 50 µg/disc
2 ; 100 µg/disc*
P - 1a
Antibacterial activity of crude methanol extract of Pseudohombus elevatus against Salmonella typhi
std - a; Amoxicillin
1 : 50 µg/disc
2 : 100 µg/disc

P - 1b
Antibacterial activity of crude methanol extract of Pseudohombus elevatus against Salmonella typhi
std - a; Gentamycin
1 : 50 µg/disc
2 : 100 µg/disc

P - 1c
Antibacterial activity of crude methanol extract of Pseudohombus elevatus against Escherichia coli
std - a; Amoxicillin
1 : 50 µg/disc
2 : 100 µg/disc
### TABLE- 26

**ANTIBACTERIAL ACTIVITY OF FISH CRUDE METHANOL EXTRACT OF *P. elevatus* AGAINST DIFFERENT BACTERIAL PATHOGENS WITH STANDARD DRUGS**

(Ampicillin, Chloramphenicol, Gentamycin and Tetracycline) 100µg/disc

Dosage of Extract - 50µg/disc & 100µg/disc

<table>
<thead>
<tr>
<th>Sl.No.</th>
<th>PATHOGENIC BACTERIAL</th>
<th>STANDARD</th>
<th>Inhibition Zone</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>50µg/disc (mm)</td>
</tr>
<tr>
<td>1</td>
<td><em>Staphylococcus aureus</em></td>
<td>Chloramphenicol</td>
<td>11</td>
</tr>
<tr>
<td>2</td>
<td><em>Vibrio cholerae</em></td>
<td>Tetracycline</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td><em>Klebsiella pneumonia</em></td>
<td>Gentamycin</td>
<td>9</td>
</tr>
<tr>
<td>4</td>
<td><em>Escherichia coli</em></td>
<td>Chloramphenicol</td>
<td>12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ampicillin</td>
<td>12</td>
</tr>
<tr>
<td>5</td>
<td><em>Salmonella typhi</em></td>
<td>Ampicillin</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Gentamycin</td>
<td>11</td>
</tr>
</tbody>
</table>