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Antimicrobial Activity of Crude Extracts Of Some Sciaenidae Fishes (Vertebrata: Actinopterigii; Perciformes) FROM PUDUCHERRY (South East Coast of India)

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ABSTRACT

Fishes have some pronounced pharmacological activities or other properties which are useful in the biomedical area. In the present study crude methanol extracts from 4 species of sciaenidae (Johinus dussumeri, Kathala axillaris, Nibea maculate and Johnius trachy cephalus), Puducherry Coastal Area were tested against five bacterial pathogens and one fungal pathogens. In bacterial activity the maximum inhibition zone 12mm was observed from Johinus dussumeri methanol extract against (Klebsiella pneumoniae). In antifungal activity maximum inhibition zone 6mm was noted from methanol extract of Nibea maculate against Candida albicans. Out the four species tested only one Nibea maculata has antifungal activity. This shows that fishes extremely strange to the fungal contact and its mediated infection. The antimicrobial property of the sciaenidae fish extracts reveals that they are low enough to bring the effect against pathogens. It may due to the incidence of bacterial presence in their habitat induce the fish to produce the antimicrobial compounds. Meager antimicrobial effect towards fungus may be of due to their rare presence in their habitat. The negative activity of other species could be attributed to the unsuitability of the method of extraction to the antibacterial screening.

Keywords: Sciaenidae - Antibacterial - Antifungal - Puducherry.

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INTRODUCTION

Sciaenidae is a family of Pisces commonly called drums, croakers. The family includes the weakfish and consists of about 275 species in about 70 genera; it belongs to the order Perciformes. They are found worldwide, in both fresh and saltwater. They are small to medium-sized, live in the benthic marine environment. The number of natural products isolated from marine organisms increases rapidly and now exceeds with hundreds of new compounds being discovered every year[1,2]. A large proportion of these natural compounds have been extracted from marine invertebrates, especially sponges, ascidians, bryozoans and molluscs and some of them are currently used in clinical trials[3]. In recent years, many bioactive compounds have been extracted from various marine animals like tunicates (ascidians), sponges, soft corals, sea hares, nudibranches, bryozoans, sea slugs and marine organisms[4,5]. The search for new metabolites from marine organisms has resulted in the isolation of more or less 10,000 metabolites[6]. The role of secondary metabolites as a chemical defence against epibiosis has been discussed[7,8,9]. Much class of bioactive compounds exhibiting antitumor, antileukemia, antibacterial and antiviral activity has reported worldwide[10].

Sciaenidae has been widely used as a traditional medicine effectively for reducing pain, inflammation and promotes wound healing in India. However, the reports of chemical components responsible to these effects are rarely found so far. The antimicrobial effect of sciaenids is rarely studied. Hence, the present study is an attempt at screening of antimicrobial potence of four sciaenid species collected from the Puducherry Coastal region, India.

MATERIALS AND METHODS

The entire research work enlisted here is done in the Kanchi Mamunivar Centre for Post-Graduate studies, Puducherry from the period of Jan'2011 – Dec'2011.

Collection and Identification

Four Sciaenidae species of Kathala axillaris (Cuvier, 1830), Johnius trachycephalus (Bleeker, 1850), Johnius dussumieri (Valenciennes, 1833), Nibea maculata (Schneidea, 1801) were collected from Solai Nagar landing centre of Puducherry (11° 46' and 12° 03'N & 79° 36'and 79° 53'E), South East Coast of India from Jan'2011 to Dec'2011.Fishes were identified based on meristic and morphometric characters with the help of FAO sheets[11].

Preparation of extracts

Samples were collected and kept in ice and brought as soon as possible to the laboratory. Body (Muscle) tissues were removed, cut into small pieces and homogenized (REMI, RQ-127A) and

extracted with petroleum ether and methanol using soxhlet apparatus for 6 hours adapted by Shiomi 1980. Then the methanolic extract was centrifuged to collect the supernatant and concentrated under vacuum in a rotary evaporator (LARK, Model: VC-100A) at low temperature. The crude methanolic extract was assayed for antibacterial and antifungal activities using standard disc diffusion method.

Microbial Cultures

Five species of bacteria and one species of fungi were used as test organisms. (Bacterial strains- Gram-positive: Staphylococcus aureus, Gram-negative: Escherichia coli, Vibrio cholerae, Pseudomonas aeruginosa, klebsiella pneumoniae). All the bacterial and fungal strains are clinical isolates, obtained from the Jawaharlal Institute of Post-Graduate Medical Education and Research, Puducherry, India.

Inoculum preparation for bacteria

Nutrient broth was prepared and sterilized in an autoclave at 15 lbs pressure for 15 min.All the 5 bacterial strains were individually inoculated in the sterilized nutrient broth and incubated at 37°C for 24 hour. Mueller Hinton Agar (MHA, Himedia) was prepared, sterilized in an autoclave at 15lbs pressure for 15 min and poured into sterile petridish and incubated at 37°C for 24 hour. The 24 hour old bacterial broth cultures were inoculated in the petridishes by using a sterile cotton swab.

Inoculum preparation for fungi

Czapek dox (Hi-media) broth was prepared and sterilized in autoclave at 15 lbs pressure for 15min. One fungal strain was inoculated in the broth and inoculated at 37°C for 72 hours. The sterilized Czapek dox agar was poured into sterile petridishes and inoculated at 37°C for 3 days. The 72 hour old fungal broth cultures were inoculated in the petridishes by using a sterile cotton swab.

Disc diffusion method

Antibacterial and antifungal activity was determined following the method of Mc Caffrey and Erdean[12] briefly, a suspension of each tested microorganism was carefully mixed in the tube containing bacterial and fungal inoculums and media for bacterial and fungal were plated separately, respective strains were cotton swabbed on petridishes. Sterile antimicrobial disc (Hi-media) was impregnated with 50 µl of crude methanolic extract of the four concentrations tested. Positive control discs containing 50 µl of tetracycline (1mg/ml) and negative control, 50 µl of methanol were used. The stocks for methanolic extracts were prepared in the concentration of 100 mg/ml. These impregnated discs were allowed to dry at laminar air flow chamber for 3 h,

and were placed at the respective bacterial and fungal plates and incubated at 37°C for 24 h for bacteria and 72 h for fungi. The diameter (mm) of the growth inhibition halos produced by the methanolic extracts of fishes was examined. Result was calculated by measuring the zone of inhibition in millimeters. All the tests were performed in triplicates.

RESULTS AND DISCUSSION

The results (Table-1) indicated that out of 4 species, 2 species exhibited antimicrobial activity against one or more of the tested micro organisms at concentration of 100µg / disc. Among the fishes tested (Figure 1) Johnius dussumieri was moderately active against klebsiella pneumoniae. The inhibition of klebsiella pneumonia (Figure 2) to the zone of 9-12 mm IZD at 100µg / disc which is comparable to the activity of standard tetracycline (19mm IZD) rest of the species does not bring any activity towards the tested strains. The antifungal activity was exhibited by methanol extract of Nibea maculata against Candida albicans. The inhibition of Candida albicans (Figure 3) to the zone of 6mm IZD at 100µg / disc which is comparable to the activity standard tetracycline (12mm IZD). But at the same time Kathala axillaris, Johnius trachycephalus, does not exhibit any activity against the tested bacterial and fungal strains.

Table-1: **Activity of four Sciaenidae species of Kathala axillaris, Johnius trachycephalus, Johnius dussumieri, Nibea maculate Body muscle tissues (100µg/disc) and Tetracycline control on the five species of bacteria (Staphylococcus aureus, Escherichia coli, Vibrio cholerae, Pseudomonas aeruginosa, klebsiella pneumoniae) and one species of fungi Candida albicans.**

Compound/Fish Species	klebsiella pneumoniae	Staphylococcus aureus	Escherichia coli	Vibrio cholerae	Proteus vulgaris	Candida albicans
Tetracycline (control)	+ + +	+ + +	+ + +	-	+ + +	+ + +
Kathala axillaris	-	-	-	-	-	-
Johnius trachycephalus	-	-	-	-	-	-
Johnius dussumieri	+ +	-	-	-	-	-
Nibea maculate	-	-	-	-	-	+

High active: + + + (inhibition zone < 12mm)

Moderately active: + + (inhibition zone 9-12mm)

Slightly active: + (inhibition zone 6-9mm)

Inactive: - (inhibition zone > 6mm).

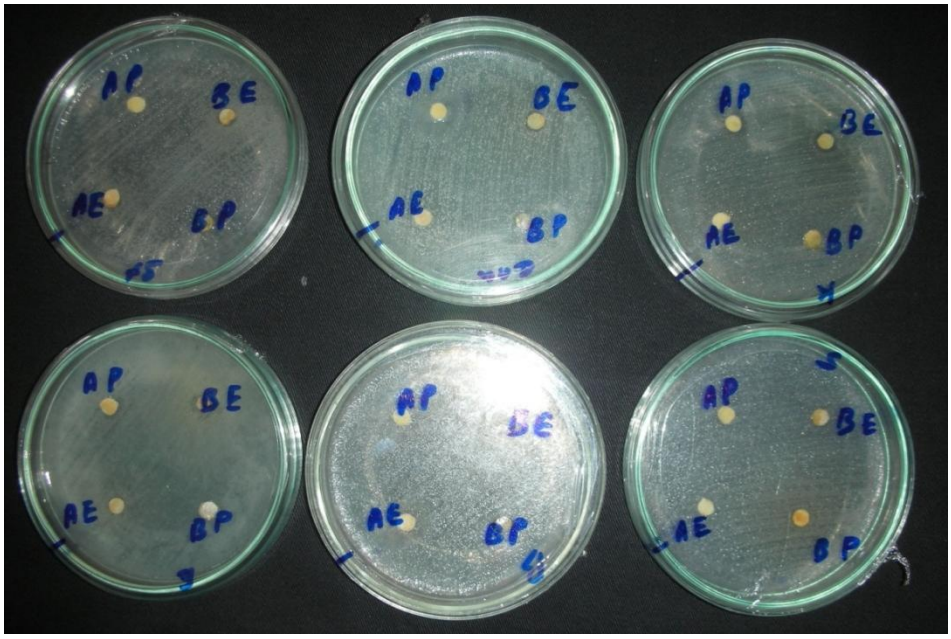

Figure 1. Antimicrobial activity of fish crude methanol extract of Johnius dussumieri and Nibea maculata against different pathogens.

AP, AE---Sample-A; crude methanol extract of Nibea maculata

BP, BE---Sample-B; crude methanol extract of Johnius dussumeri

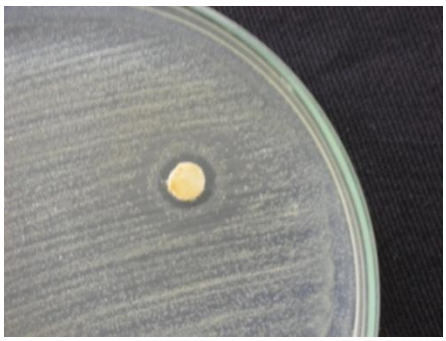

Figure 2. Antibacterial activity of fish crude methanol extract of Johnius dussumieri against klebsiella pneumonia (Enlarged).

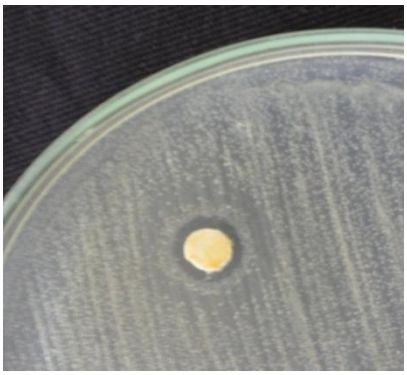

Figure 3. Antifungal activity of crude methanol extract of Nibea maculata against Candida albicans.

In recent years, great attention has been paid to study the bioactivity of natural products and their potential pharmacological utilization. The rationale of searching for drugs from marine environment stems from the fact that marine plants and animals have adapted to all sorts of marine environments and these creatures are constantly under tremendous selection pressure including space competition, predation, surface fouling and reproduction. Many of these organisms have been antimicrobial properties, although most of the antibacterial agents that have been isolated from marine sources have not been active enough to compete with classical antimicrobials obtained from micro organisms[13].

Many antimicrobial proteins have been isolated from plants and animals, from insects to mammals. These proteins are predicted to operate as a first-line host defense mechanism, acting against pathogenic bacteria, fungi and other parasites. Generally nonspecific but rapidly active during a parasite invasion, they constitute host defenseless costly than antibodies. The number of compounds isolated from various microorganisms has increased the intensity and now exceeds 10,000 [14] with hundreds of new compounds still being discovered[1]. The marine environment holds an unprecedented number of unusual chemical structural glasses, even with activity against HIV[15].

Antimicrobial proteins are an important part of the innate immune system. They have received only scant attention in fish[16]. The potentiality of marine fishes as a source of biologically active products is largely unexplored. The skin acid extracts of channel catfish Ictalurus punctatus exhibited antimicrobial activity against Aeromonas[17] isolated a novel glycosylated protein from the crude epidermal mucus extract of tench Tinca tinca, eel Anguilla anguilla and rainbow trout Oncorhynchus mykiss showed antimicrobial activity against both gram –ve and gram +ve bacteria. The crude methanol extract of mucus of Narcine timlei showed antibacterial activity against Vibrio cholerae, salmonella typhimurum, Shigella flexneri[18]. In the present study the moderate antibacterial activity was brought out by the Johnius dussumeri against klebsiella pneumoniae. The future prospective of this research mission is facing towards the analysis of possible organic compounds present in the fish extract and compound purification and isolation will be attempted in order to bring the exact target moiety, which is responsible for bringing the antimicrobial effect against the tested pathogenic organisms.

CONCLUSION

Out the four species tested only one Nibea maculata has antifungal activity. This shows that fishes extremely strange to the fungal contact and its mediated infection. The antimicrobial

property of the sciaenidae fish extracts reveals that they are low enough to bring the effect against pathogens. It may due to the incidence of bacterial presence in their habitat induce the fish to produce the antimicrobial compounds. Meager antimicrobial effect towards fungus may be of due to their rare presence in their habitat. The negative activity of other species could be attributed to the unsuitability of the method of extraction to the antibacterial screening.

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