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MICROBIOLOGICAL STUDY ON THE EXTRACTS OF THE ASCIDIAN *EUDISTOMA VIRIDE*

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ABSTRACT

Ascidians have some pronounced bioactive agents which shows very useful in the field of antimicrobial theraphy. In the present study methanol, dichloromethane, chloroform and hydroalcohol extracts from the ascidian *Eudistoma viride* were tested against various gram positive and gram negative microbial species. The methanolic extract of *Eudistoma viride* showed maximum activity against *Staphylococcus aureus, Escherichia coli & Salmonella typhi* and their zone of inhibition, respectively (13± 1.24, 12 ± 1.13 & 11 ± 0.92). The dichloromethane extract of *Eudistoma viride* showed maximum activity against *Bacillus subtilis & Staphylococcus aureus*, zone of inhibition (9.5 ± 0.67). The MIC values of the methanolic extracts of *Eudistoma viride* reported very low when compared with the other crude extracts.This result suggest that the antibacterial activity shown by *Eudistoma viride* was concentration dependent and can be used as effective inhibitor in the field of life science.

Key words: Anti-microbial, Minimum inhibitory concentration, Ascidian

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INTRODUCTION

The number of natural products isolated from marine organisms increases rapidly, and now exceeds. With hundreds of new compounds being discovered every year, 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 in clinical trials (1). The need for discovery of new and novel antibiotics is imperative because evidence suggests that

development and spreads of resistance to any new antimicrobial agents is inevitable. It has been widely demonstrated that the colonial ascidians are rich in bioactive substances (2). The case of living marine surfaces the colonization process can additionally be affected by organic metabolites produced by the host organism. These metabolites may affect bacteria in a number of ways, ranging from the induction of a chemo tactic response to the inhibition of bacterial grow or cell death (3). The role of secondary metabolites as a chemical defense against epibiosis has been discussed (4-6). The aim of the present study was, first to analyze the antibacterial activity from the tissue extracts of ascidian *Eudistoma viride* were tested against different pathogenic bacterial strains.

Collection and Authentication

Bulk samples of Ascidian *Eudistoma viride* was collected as common and persistent biofoulants from a depth 1- 2 m along Tuticorin coastal waters (Lat.8° 47' 20" and Long. 78° 09' 70"), southeast coast of India by snorekeling between May and June, 2007. The samples were thoroughly washed with sea water and cleaned of sand, mutt and overgrowing organisms at the site of collection and was immediately transported to laboratory and the collected specimen no Tun / 2314 was identified by following standard keys and confirmed by H. Abdul Jaffar Ali, Department of Biotechnology, Islamiah College, Vaniyambadi, India. A voucher specimen of this ascidian was maintained at the biotechnology lab at Vels College of pharmacy for reference.

Preparation of Extracts

The animals were rinsed with sterile distilled water, weighed, cut into small pieces. Extraction of bioactive compound from the tissue sample was done with methanol, dichloromethane, chloroform and hydroalcohol. Samples were then soaked in the above mentioned solvents for 48 hrs and filtered with Whatman No.1 filter paper and evaporated to dryness in a vacuum rotary evaporator and the residue reconstituted in an appropriate buffer before testing (7). Further the extracts were used for microbiological studies.

Micro-organisms and Culture Media

Standardized cultures of three Gram-positive bacterial species *Bacillus subtilis* (ATCC 9372), *Staphylococcus aureus* (ATCC 13709), *Bacillus cereus* (ATCC 10987) and three Gram-negative bacterial species *Escherichia coli* (ATCC 9637), *Klebsiella aerogenes* (ATCC 9621), *Salmonella typhi* (ATCC 19430) were obtained from the Department of Biotechnology, Vels college of pharmacy, Chennai. These organisms were stored on nutrient agar slants which were enriched with 7% blood and kept at 4°C prior to use for antimicrobial susceptibility tests. Nutrient agar (M090) and nutrient broth (M002) were obtained from Himedia Laboratories (Mumbai).

Disc Diffusion Method.

Antibacterial activity was carried out by using standard disc diffusion method (8) . Whatman No. 1 filter paper disk of 6-mm diameter was sterilized by autoclaving for15 min at 121°C. The sterile disks were impregnated with different extracts (30 µg/ml). Agar plates were surface inoculated uniformly from the broth culture of the tested microorganisms. In all cases, the concentration was approximately 1.2x10 ^{8}CFU/ml (9). The impregnated disks were placed on the medium suitably spaced apart and the plates were incubated at 37°C for 24 h. Disk of Ciprofloxacin (30µg/ml) was used as a positive control. The diameter (mm) of the growth inhibition halos caused by the extracts of marine organisms was examined. All the assays were carried out in triplicate (10).

Minimum Inhibitory Concentration(MIC)

The MICs of the extracts were determined for the test organisms in triplicates by using broth dilution technique as recommended by European Committee for antimicrobial susceptibility testing (EUCAST. E. Dis. 5.1) with reference to ciprofloxacin. Tests were performed in a set of eight sterilized tubes with nutrient broth medium capped with cotton plugs. The test compound was dissolved in dimethylsulfoxide (512 µg/ml) and serially diluted in tubes from 1 to 8. Aliquots (1 ml) of the standard broth culture were added to 1 ml of each serially diluted test solutuion. The procedure was repeated on the test organisms using the standard antibiotic (Ciprofloxacin). A tube containing nutrient broth only was seeded with the test organism as described above to serve as control. Capped tubes with cotton plugs were incubated at35 –

37ºC for 20 h in comparison to standard 0.5 McFarland reagent After incubation, the tubes were then examined for microbial growth by observing for turbidity (11).

Statistical Analyses of Data

The experiments were laid out according to randomised block design (for single factor experiments) or nested design (two factor experiments). In Each zone of inhibition experiments usually had three replicates and the mean of three replicates was noticed. The analysis of variance (ANOVA) appropriate for the design was carried out to detect significance of differences among the treatment means.

Results

The antimicrobial activity of (methanol, dichloromethane, chloroform and hydro alcohol extracts) of *Eudistoma viride* against gram positive bacterial strains *Bacillus subtilis Staphylococcus aureus, Bacillus cereus* and three Gram-negative bacterial strains *Escherichia coli, Klebsiella aerogenes, Salmonella typhi* is shown in the Fig 1. The positive controls showed inhibition diameters ranging from 10 - 17 mm (Ciprofloxacin). The methanolic extract of *Eudistoma viride* showed maximum activity against *Staphylococcus aureus, Escherichia coli & Salmonella typhi* and their zone of inhibition, respectively (13± 1.24, 12 ± 1.13 & 11 ± 0.92). The dichloromethane extract of *Eudistoma viride* showed maximum activity against *Bacillus subtilis & Staphylococcus aureus,* zone of inhibition (9.5 ± 0.67). MIC of methanol, dichloromethane, chloroform and hydro alcohol extracts of *Eudistoma viride* was also carried out against all the bacterial strains mentioned above by broth dilution technique as recommended by European Committee for antimicrobial susceptibility testing (EUCAST. E. Dis. 5.1) with reference to ciprofloxacin as shown in Fig 2. The MIC values of the methanolic extracts of *Eudistoma viride* was very low against *Bacillus subtilis Staphylococcus aureus, Bacillus cereus* & *Escherichia coli* showed 8µg/ml and it showed 16µg/ml against *Salmonella typhi* . 8µg/ml of the dichloromethane extract of *Eudistoma viride* was the lowest MIC against *Klebsiella aerogenes.* The antibacterial activity shown by *Eudistoma viride* was concentration dependent as shown in Fig 1&2.

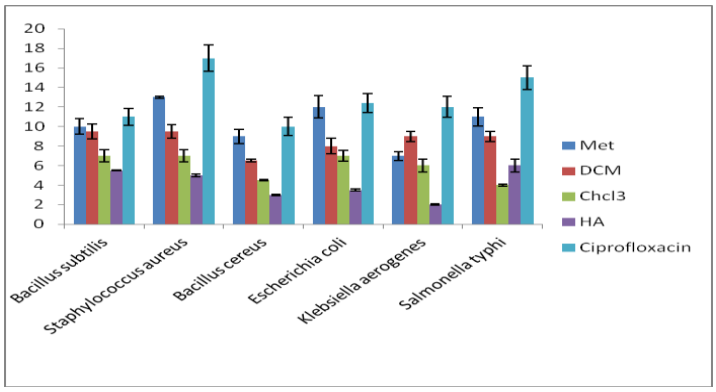

Fig1: *In vitro* antibacterial activity zone of inhibition mm/µg/ml of extracts of *Eudistoma viride*

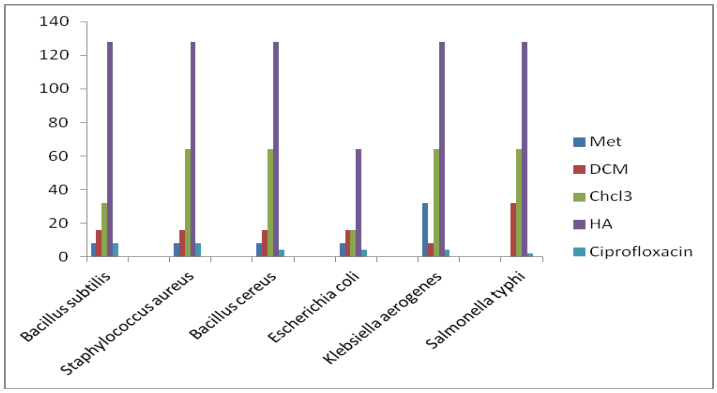

Fig 2: *In vitro* antibacterial activity MIC µg/ml of extracts of *Eudistoma viride*

Discussion

Attempt to locate antimicrobial activity in marine organisms was initiated around 1950's (12). Since this time large numbers of marine organisms from a wide range of phyla have been screened for antimicrobial activity (13). Many of these organisms have been showing antimicrobial properties, although most of the antibacterial agents that have been isolated from the marine source have not been active enough to complete with classical antimicrobial obtained from microorganisms (14). To derive a pronounced antibacterial activity the present investigation of *Eudistoma viride* has been monitored against some bacterial strains. The maximum activity was recorded in methanol extract against various bacterial strains. Methanol extracts of *Eudistoma viride* showed the highest activity against *Bacillus subtilis Staphylococcus aureus, Bacillus cereus, Klebsiella aerogenes* & *Escherichia coli,* dichloromethane extract showed the highest against *Klebsiella*

aerogenes. From the above studies, it is concluded that the marine ascidians may represent new sources of anti-microbial with stable, biologically active components that can establish a scientific base for the use of tunicates in modern medicine.

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