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Screeing of antimicrobial activity of the extracts from the selected marine sponges in a*urora globostellata* (carter) and *spirastrella inconstans* var. *moeandrina* dendy from tuticorin region

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ABSTRACT

The antibacterial activity of the ethyl acetate extracts of two marine sponges collected from Tuticorin gulf of mannar region was tested against six human pathogenic bacteria and five human pathogenic fungi using the agar disk diffusion method. Bacteria cultures, *Vibrio cholarae, Escherichia coli, Salmonella typhi , Pseudomonas aeruginosa , Klebsiella pneumonia* (Gram negative bacteria), *Bacillus subtilus, Staphylococcus aureus* (NCIM 2079). Sponge extracts of *Aurora globostellata* (Carter) and *Spirastrella inconstans* Var. *moeandrina* Dendy appeared to be quite promising due to their capacity to inhibit the growth of *Pseudomonas aeruginosa* and methicillin resistant strains *Staphylococcus aureus* and *E.coli* as well as a broad-spectrum activity against all the other bacteria. The highest activity was obtained for the ethyl acetate extract of *Aurora globostellata* (Carter) against the Gram-positive bacteria *Klebsiella pnemoniae* (inhibition zone 12.33±0.33 mm) and *Salmonella typhi* (14.00±0.57) and against *C. albicans* (12.66±0.66 mm), *T. rubrum* (12.33±0.33 mm) and *Aspergillus flavus (*14.33±0.33). This extract is currently undergoing further analysis to identify the active compounds. These promising results in relation with antibacterial and antifungal activity in vitro open the way for complementary investigation in order to purify and identify active molecules.

Introduction

Marine invertebrates are a rich source of biologically active secondary metabolites. The biosynthesis of secondary metabolites by these invertebrates has been speculated as a result of their physical and biochemical adaptation to their environment. In the last two decades, many new compounds were isolated from these organisms and have been promoted as candidates for the development of new drugs, especially as anti-cancer drugs. There is therefore, a need to continue research for discovery of novel secondary metabolites from marine invertebrates. The largest groups of marine invertebrates as a source of secondary metabolites are the sponges. The structurally diverse varieties of metabolites have high therapeutic potential to treat human diseases and have made them worthy of research for marine natural product chemists (Ireland *et al.*, 1993).

Marine sponges are shown to exhibit antibacterial, insecticidal, antiviral and antiplasmodial activities (Compagnone RS, et al., 1998). Antifungal activity of *Haliclona spp*. against *Aspergillus* strains has also been reported (Faulkner DJ, 2001). The ocean covers about 70 % of the earth surface providing a diverse living environment for invertebrates (Lalli and Parsons, 1993). Therefore, marine natural products will play a major role in drug discovery in the future. The work on marine natural products started 54 years ago when Bergman discovered the novel bioactive arabino-nucleoside from the marine sponge *Cryptotethya crypta* (Bergman and Feeney, 1951).

In order to survive in a highly competitive environment, marine invertebrates produce a tremendous diversity of extreme toxic compounds. This has stimulated research groups to screen marine samples in various cytotoxicity assays. Marine natural products evaluations were mostly focused on anti-cancer and anti-inflammatory activity. Some of the compounds from marine invertebrates initially discovered were either too toxic or not effective in treating diseases for pharmaceutical purposes, but were found to be useful as biological tools or as cosmetic ingredients or as agrochemicals (Fenical, 1997).

The extract from this gorgonian shows anti-inflammatory activity, which nowadays is used as an ingredient in cosmetic skin care products (Proksch, *et al.*, 2002). Biological tools or biochemical properties have contributed to the understanding of human diseases. Compounds (in case of pharmacological probes) that have high potential to reveal the biochemistry of diseases could be used as biological tools. This is exemplified by ziconotide, a peptide produced by *Conus* mollusk, which potentially blocks the calcium channel. This compound inhibits neurotransmitter release from incoming sensory fibers and spinal cord neurons further transmitting the signal to the brain (Olivera, 2002). There are many classes of alkaloids which were isolated from marine sponges. However, one interesting group is the bromopyrrole-imidazole alkaloids due to its biological activities and structural diversity. About 90 compounds of this class of alkaloids were characterized (Hoffmann and Lindel, 2003). A variety of antimicrobial substances have been isolated from various species of marine sponges (Zaro BA, 1982). In this

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report, we describe the biological effect of ethyl acetate extracts of two marine sponges collected from the coastline of Tuticorin region for their antimicrobial activities against six human pathogenic bacteria and five human pathogenic fungi in order to find new antibacterial and antifungal metabolites.

Material and Method

Sponge collection

The sponge samples were collected by scuba during scientific expedition in a rocky slope at water deeper than 20 m depth from the coastal water of gulf of mannar, Tuticorin region (March 2010). The sponge specimens were cleaned and stored deep freezer at - 20^0C until used in extraction.

Preparation of extracts

Each sponge sample (500 g wet weight) was cut into small pieces mixed with a blender and macerated at 4^0C during 48 h. The macerate was lyophilised during 3 days using a laboratory freeze dryer (Christ). The lyophilised material was subjected to an extraction of biologically active components which were carried out with different solvents in the order of increase polarity: hexane, ethyl acetate and methanol by soaking at ambient temperature. The residues (crude extracts) thus obtained were finally dried under rotary vacuum evaporator and screened against six human pathogen bacteria and four human pathogen fungi using the agar disk diffusion method. (Selvin J and Lipton AP, 2004).

Screening for antibacterial activity of sponge extracts

For the antimicrobial screening 6 species of bacterial isolates and five species of fungal isolates were selected. The bacterial and fungal strains were obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. *Vibrio cholarae, Escherichia coli, Salmonella typhi* , *Pseudomonas aeruginosa* , *Klebsiella pneumonia* (Gram negative bacteria), *Bacillus subtilus, Staphylococcus aureus* (NCIM 2079), (Gram positive bacteria) strains were used. The antibacterial activity of sponge extracts was performed using the agar-disk diffusion assay. The bacterial cultures were first grown on Muller-Hinton infusion agar (MHI) plates at 37^0C for 18 to 24 h prior to seeding onto the nutrient agar. One or several colonies of similar morphology of the respective bacteria were transferred into API suspension medium (Biome´rieux) and adjusted to the 0.5 McFarland turbidity standard with a Densimat. The inocula of the respective bacteria were streaked into the MHI agar plates using a sterile swab and were then dried. A sterile filter disk 6 mm in diameter (Whatman paper No. 1) was placed on the infusion agar seeded with bacteria and 10 ml of the ethyl acetate extract was dropped onto each paper disk (5 mg/disk) by first applying 5 ml with the pipette.

Screening for antifungal activity of sponge extracts

Candida albicans, T. rubrum, Fusarium species , *Aspergillus flavus* and *Aspergillus níger* mold fungi were used as fungal test microorganisms were used. Screening for the antifungal activity of the extracts was performed using the agar-disc diffusion method as previously described. All fungal cultures were first grown on Sabouraud chloramphenical plate at 30^O C for 18-24 h prior to inoculation onto the nutrient agar. Several colonies of similar morphology of the clinical fungi were transferred into API suspension medium and adjusted to 2 McFarland turbidity standard with a densimat. The inocula of the respective fungi were streaked on to Sabouraud chloramphenical agar using a sterile swab and then dried. A sterile filter disc, diameter 6 mm (Whatman paper No. 1) was placed. An amount of 10 ml of extract was dropped on each paper disc (10 mg/disc) as previously described. The treated Petri dishes were placed at 4^0C for 1-2 h and then incubated at 37^0 C for 18-24 h.

Results and Discussion

Aqueous extracts from the seven sponges shown a very weak antibacterial activity compared to the ethyl acetate extracts (data not shown). As shown in Table 1, ethyl acetate extracts from different sponges are active at least against one of the bacterial strains. Ethyl acetate extract from *Aurora globostellata* (Carter) exhibited significant activity against all bacterial strains. An important activity against the bacterial strains is also observed with the organic extract of *Aurora globostellata* (Carter) except its weak activity against *E.coli and Staphylococcus aureus.* In this present investigation, as mentioned, *Spirastrella inconstans* Var. *moeandrina* Dendy inhibited E. coli and Staphylococcus aureus considerably; this indicates that marine sponges remain an interesting source of new antibacterial metabolites with better activity then some antibiotics.

Fungi especially *T.rubram* and *A. niger* are more resistant than *C. albicans (11.33*± 066) and *Aspergillus flavus* (14.33±0.33) (Table 2). *A. globostellata* were highly sensitive to all the microbes tested. The two sponges belonged to the species *S. inconstans* var. moendrina were good antimicrobial agents. *Staphylococcus aurues* (15.33± 0.66 mm) when compared to the inhibitory role of all the other extracts.

Antifungal activity was also observed in the extracts of the sponges. *Candida albicans and A. níger* was also showed resistance to the ethyl acetate. The presence of chemical constituents like steroids, Tri terpenoids, Reducing sugar, Alkaloids, phenolic compound, Saponin, Xantho protein, Tannin, Flavanoids and Aromatic acid, were tested in the selected two species of sponges.

On the other hand, the development of resistance to current antibacterial continues to be a serious difficulty in the treatment of infectious diseases, and therefore the development of new antibiotics has become a high priority in biomedical research (Meylan A, 1988). In addition, the frequency of invasive fungal infection has risen substantially with the increasing numbers of immunocompromised patient, such as those infected with HIV, receiving cancer chemotherapy, immunosuppressive therapy, or broad-spectrum treatment (Faulkner DJ, et al., 2000).

Figure :1 **Figure : 2**

Conclusion

Sponges species collected from Tuticorin coast have been shown to possess a specific antibiotic activity from some fractions. The most interesting species are *Aurora globostellata* (Carter) and *Spirastrella inconstans* Var. *moeandrina* Dendy. These observations corroborate the importance of both genera Aurora and Spirastrellata as a potential source for potential source for biological active compounds such as antibacterial (Bakkestuen AK, et al., 2005) antiviral (Da Silva AC, et al.,2006) and antitumoral (Ferretti C, et al., 2007) substances. Furthermore, the encouraging biological activities seen in this study show that the Tunisian coastline is a potential sour

of sponge species worthy of further investigation.

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Table 1. Antibacterial activity of ethyl actate (EtoAc) extract of two species of sponges collected from Gulf of Mannar Tuticorin region

Sponges	Inhibition zone diameter (mm) for different microorganisms					
	Escherichia coli	*Salmonella typhi*	*Pseudomonas aeruginosa*	*Klebsiella pneumonia*	*Staphylococcus aureus*	*Bacillus subtilus*
Aurora globostellata	15.33±0.33	13.33±0.66	11.66±0.33	11.66±0.33	15.33±0.66	10.66±0.66
Spirastrella inconstans var. moeandrina	15.33±0.66	14.00±0.57	12.33±1.20	12.33±0.33	14.00±0.57	11.66±0.33

Table 2. Antifungal activity of ethyl acetate (EtoAc) extracts of two species of sponges collected from gulf of Mannar Tuticorin region

Sponges	*Saccharomyces cerevisiae*	***T.rubrum***	***Fusarium species***	*Candida albicans*	*Aspergillus flavus*
Aurora globostellata	9.00±0.57	12.33±0.33	8.33±0.88	12.66±0.66	14.33±0.33
Spirastrella inconstans var. moeandrina	10.33±0.33	11.00±0.57	8.66±0.66	11.33±0.66	11.66±0.33