Screening of the selected marine sponges from the coasts of Turkey for antimicrobial activity

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Received 16 June 2016; revised 03 January 2017

In the course of our program to search for bioactive compounds from the marine sources, the methanol extracts of 8 marine sponges were screened for their antimicrobial activity. Sponges were extracted with methanol and solved in dimethylsulphoxide (DMSO) at a final concentration of 512 µg ml-1 for preparing stock solutions. Antibacterial and antifungal activity tests were carried out against standard and clinical isolates with microdilution method as described in CLSI standards. The extracts of sponge species collected from coasts of Turkey have been shown to possess antibacterial and/or antifungal activity.

Keywords: Antimicrobial activity; Coasts of Turkey; Marine; Sponges

Introduction

In recent years, a large number of studies have been conducted searching the antimicrobial activity of natural products. Although the most important sources of antimicrobial substances are the terrestrial microorganisms (fungi, actinomycetes and bacteria) and medicinal plants, however, marine organisms also present a big source of secondary metabolites. Reports showed that more than 17000 different marine natural products have been discovered, 10000 in the last two decades alone and approximately 10-15 different marine natural products are currently in clinical trials, mostly in the areas of cancer, pain or inflammatory diseases1,2. Among the groups of marine invertebrates, the sponges (Phylum Porifera) are receiving much attention mainly because of the unique natural compounds (unusual nucleosides, terpenes, sterols, peptides, alkaloids, fatty acids, peroxides and amino acid derivatives) characterized by a huge variety of biological activities such as; antioxidant, antiviral, antibacterial, antifungal, antitumor, anticancer, antifouling activities which acts as potential natural compounds of interest for pharmaceutical applications3-5.

Many species of sponges are long lived and resistant to the bacterial decomposition. They are expected to produce antimicrobial compounds. This was found to be true, the extracts of different sponges showed broad spectrum antibiotic activity. Some of the extracts are especially active against Staphylococcus, Pseudomonas, and pathogenic yeasts. It was found that bromine compounds responsible for broad antibiotic activity. According to Laport et al., marine sponges are among the most promising sources of new antimicrobial substances6.

Turkey is a country of peninsula surrounded by the Black Sea at the north, the Aegean Sea at the west, and the Mediterranean Sea at the south. Despite of its long coastal line, which is 8400 km in total, there have been limited works on its marine prosperity from the view point of isolation of biologically active compounds and also few studies investigated the composition of bacterial communities associated with sponges in Turkey7.

In the course of our program to search for bioactive compounds from the marine sources, the methanol extracts of 8 marine sponges were screened for their antimicrobial activity. Present study was to investigate the antimicrobial activity spectrum of the marine sponges extracts collected from the different coasts of Turkey.

Materials and Methods

The sponges (Figure 1) were collected from given locations in Table 1, at depths varying from 10-30 m
Fig. 1a to 1h — Marine sponge samples from coasts of Turkey [1a - Agelas oroides; 1b - Axinella polypoides; 1c - Axinyssa digitata; 1d - Calyx nicaeensis; 1e - Dictyonella incisa; 1f - Dysidea avara; 1g - Haliclona aquaeductus (kindly provided by Tahsin Ceylan); 1h - Sarcotragus spinulosa] according to the seasons.
by scuba diving and transferred immediately to the laboratory in Ankara while kept in ethanol (70%) during transfer and later on put in deepfreeze until the experimental process. Sponges were collected and identified by marine biologist Dr. Bulent Gozcelioglu based on conventional macroscopic and microscopic marine sponge identification procedures. Sponge samples were deposited at Ankara University, Faculty of Pharmacy, Ankara, Turkey.

Sponges were cut into small pieces and extracted with methanol (3x200 ml) solution was filtered and solvents were removed by rotary vacuum evaporator under reduced pressure so as to get the crude methanol extract. The concentrated crude extract was kept in the refrigerator for further use7.

The stock solutions of sponge extracts were prepared in dimethylsulphoxide (DMSO) at a final concentration of 512 µg mL-1 and sterilized by using 0.22 µm Millipore Membrane Filter (MA 01730, USA).

Antibacterial activity tests were carried out against standard and clinical isolate (obtained from Department of Microbiology, Gulhane Military Medical Academy, Ankara, Turkey) strains which are especially resistant to known antibiotics and previously not tested with these sponge extracts. The strains of gram-negative bacteria; Acinetobacter haemolyticus ATCC 19002, Acinetobacter septicum (GATA Microbiology - NRB 239), Klebsiella pneumoniae CDC 529, the strains of gram-positive bacteria; Staphylococcus aureus JCSC 4744, Staphylococcus epidermidis ATCC 35984 and the strains of yeasts; Candida glabrata ATCC 90030, Cryptococcus neoformans NIH 68 and Cryptococcus gattii NIH 112 were employed for determination of antibacterial and/or antifungal activity of these eight sponge extracts.

Antimicrobial susceptibility testing was performed by a modified microdilution method as described in CLSI M07-A9 standard for bacteria and CLSI M27-A3 standard for yeasts8,9. The tested two fold serial dilutions of the extracts were between 256 and 0.5 µg mL⁻¹. The sealed microplates were placed in a humid chamber and incubated at 35°C for 24 and 48 hours for bacteria and yeasts, respectively. Lowest concentration of the extract that completely inhibited macroscopic growth of the microorganism was accepted as minimum inhibitory concentration (MIC).

**Results and Discussion**

The sponge species of Agelas oroides, Axinella polypoides, Axinysa digitata, Calyx nicaeensis, Dictyonella incisa, Dysidea avara, Haliclona aquaeductus and Sarcotragus spinulosus were isolated and identified in the study. Table 2 shows in vitro antimicrobial activity of five bacteria and tree yeasts against this eight sponge extracts and a sampleimage of microdilution method was given in Figure 2.

There was macroscopic growth in all positive control wells accordingly solvents and/or medium did not have any effect on bacteria and yeasts. At the same time there was no growth in negative control wells.

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**Table 1 — Sponge species and diving locations**

<table>
<thead>
<tr>
<th>Species Name</th>
<th>Location</th>
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<tbody>
<tr>
<td>Agelas oroides</td>
<td>Fethiye (36°35'58&quot;N 29°1'36&quot;E)</td>
</tr>
<tr>
<td>Axinella polypoides</td>
<td>Gökova (37°2'29&quot;N 28°1'97&quot;E)</td>
</tr>
<tr>
<td>Axinysa digitata</td>
<td>Ayvalik (39°19'22&quot;N 26°32'6&quot;E)</td>
</tr>
<tr>
<td>Calyx nicaeensis</td>
<td>İzmir (38°30'8&quot;N 26°38'29&quot;E)</td>
</tr>
<tr>
<td>Dictyonella incisa</td>
<td>Seferihisar (38°10'49&quot;N 26°45'43&quot;E)</td>
</tr>
<tr>
<td>Dysidea avara</td>
<td>Ayvalik (39°19'46&quot;N 26°35'57&quot;E)</td>
</tr>
<tr>
<td>Haliclona aquaeductus</td>
<td>Gölcük (40°43'28&quot;N 29°49'53&quot;E)</td>
</tr>
<tr>
<td>Sarcotragus spinulosus</td>
<td>Amfora (39°20'35&quot;N 26°35'9&quot;E)</td>
</tr>
</tbody>
</table>

**Table 2 — Minimum inhibitory concentrations (µg mL⁻¹) of the extracts against tested microorganisms**

<table>
<thead>
<tr>
<th>Extract of</th>
<th>Microorganisms</th>
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<tr>
<td></td>
<td></td>
</tr>
<tr>
<td>A. oroides</td>
<td>32 16 64</td>
</tr>
<tr>
<td>A. polypoides</td>
<td>32 16 64</td>
</tr>
<tr>
<td>A. digitata</td>
<td>32 16 64</td>
</tr>
<tr>
<td>C. nicaeensis</td>
<td>32 32 64</td>
</tr>
<tr>
<td>D. incisa</td>
<td>32 32 64</td>
</tr>
<tr>
<td>D. avara</td>
<td>32 16 32</td>
</tr>
<tr>
<td>H. aquaeductus</td>
<td>32 32 64</td>
</tr>
<tr>
<td>S. spinulosus</td>
<td>8 16 64</td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>SA: 16 16 16</td>
</tr>
<tr>
<td></td>
<td>SE: 16 16 16</td>
</tr>
<tr>
<td></td>
<td>CG: 16 32 16</td>
</tr>
<tr>
<td></td>
<td>CrN: 16 16 8</td>
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<tr>
<td></td>
<td>CrG: 32 32 8</td>
</tr>
</tbody>
</table>
and chronic systemic infections. For these reasons researches of anti-biofilm featured catheter increasing nowadays. Maybe a new product has been found derived from marine sponges for producing anti-biofilm featured catheter.

Marine sponges are a rich source of bioactive compounds, and many species can be useful for the development of new antimicrobial drugs. Screenings of marine sponges for antimicrobial activity led to the isolation and characterization of nearly 800 antibiotic compounds have been isolated from marine sponges. In the continuing research by sponges two compounds selected as promising leads for preclinical assessment, lasonolides (antifungal activity) and psammaplin A (antibacterial activity)6,17.

Up to date, there have been many reports on secondary metabolites of the marine sponges of genus Agelas, Axinella, Dysidea, Haliclona and Sarcotragus, which are analyze in this study7,18-21. Agelas species have been reported to have bromopyrrole type alkaloids and fatty acids in major compounds7,22,23. Antimicrobial bromopyrrole alkaloids, oroidin and nagelamides Q, R, J, K, L, M and N have been isolated from Agelas and Axinella species have been reported to contain various terpene derivatives, alkaloids, cyclopeptides, polyketides, etc23-25. The example of antimicrobial curvularin and α, β-dehydrocurvularin from Eupenicillium sp. associated with the marine sponge Axinella sp.26. Urban and his colleagues isolated imidazole alkaloids Axinellamines B-D from same Australian marine sponge species18.

Dysidea species have been one of the most chemically investigated species since 1990’s which were isolated avarol and avarone, marine sesquiterpenes sulphates. They have different pharmacological activities including anti-inflammatory, anti-sporiotic and antibacterial activity. Antimicrobial alkaloids (dysideanins A and B) also isolated from South China Dysidea sp.19,27-30.

The scientific literature contains numerous examples of antimicrobial compounds from that genus Haliclona sp. including alkylpipеридine, bromopyrrole and pyrroloiminoquinone alkaloids. Examples of antimicrobial alkaloids from Haliclona sp. included halicyclamine A, haliclonacyclamine A and B which exhibited antibacterial activity against diverse microbial strains20,31. Lee et al. isolated eight novel cyclic bis-1,3-dialkylpyridiniums (antimicrobial) from the sponge Haliclona sp.32.
Sponges from the genus *Ircinia* are the most widely studied in terms of their secondary metabolites. A number of different compounds have been isolated and characterized, many of them being antimicrobial. In recent years a number of scientific studies provided evidence of four hydroxylated polyprenylhydroquinones and furanosterterpenes tetronic acids have been isolated from *Sarcotragus spinosulus* (under the name Ircinia spinosa). They have been shown to have an enhanced antibacterial, anti-inflammatory and cytotoxic activity\(^1\)\(^2\)\(^3\)\(^4\).

Some anti-inflammatory agents isolated from *Dictyonella incisa* in the last three decades but an antibacterial agent has not been found yet from *Dictyonella* species\(^5\)\(^6\). Perhaps immune defense mechanism of this sponge species, steroids play an effective role adequately.

Many studies in the last few years discovered sesquiterpenes (monocyclic bisabolene, bicyclic eudesmane, cadene, gorgonane, tricyclic pupukenane, cubebene, and aromadendran) from genus *Axinyssa* that were classified for their cytotoxic, antimicrobial, anthelmintic, antimalarial and antifouling properties\(^7\)\(^8\).

Calysterol is the best known steroid which has been isolated from *Calyx nicaeensis*. Another steroids and stanols have been isolated from *Calyx* species especially *Calyx nicaeensis* but an antibacterial or antifungal agent has not been discovered yet. Perhaps some stanols result from bacterial metabolism of the endogenous sponge sterols\(^9\).

**Conclusions**

The major drawback of this preliminary study was that we have not identified the active compound. However, all crude extracts obtained from eight species of sponge tested in this study showed considerable antimicrobial activity against multidrug resistant bacteria and yeasts that cause serious health problems in humans.

**Acknowledgement**

This work was partially assisted by FP-7 IRSES BACT 295226.

**References**


