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# Antifungal and Antibacterial Activities of Three Marine Sponges Obtained From the Gulf of Saros in Turkey

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## ABSTRACT

*In vitro* antimicrobial activity of methanol extracts from *Spongia officinalis*, *Spongia agaricina*, *Aplysina aerophoba* collected from the Gulf of Saros in Turkey was screened against sixteen microorganisms in this study. The antimicrobial activity was determined using agar disc diffusion method. *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25902, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 12228, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Aspergillus niger*, *Aspergillus fumigatus* var. *elipticus*, *Aspergillus flavus*, *Penicillium granulatum*, *Penicillium rugulosum*, *Penicillium jenseii*, *Aspergillus candidus*, *Geotrichum candidum* and *Candida albicans* ATCC 10239 were used as test microorganisms. Zones of inhibition of the extracts were compared with different standard antibiotics like Ampicillin and Penicillin for antibacterial activity and Ketoconazole and Nystatin for antifungal activity. The results showed that especially *Aplysina aerophoba* and *Spongia agaricina* remarkably inhibited both bacterial and fungal growth. While antimicrobial activity was obtained from the extract of the *Aplysina aerophoba* against the Gram-positive bacteria, the Gram-negative bacteria and some fungi, *Spongia agaricina* extract exhibited antimicrobial activity against all of the test microorganisms excluding one bacterium. Hence, it has been demonstrated that a new bioactive natural product can be discovered from the two sponges in this study

**Keywords:** Marine sponges, antimicrobial activity, disc diffusion method, test microorganisms

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## INTRODUCTION

Plants have been used for medical purposes for thousands of years. Currently, data on the

antimicrobial activity of numerous plants have been scientifically confirmed on pathogenic microorganisms resistant to antimicrobials. However, the number of the investigations on marine organisms is more limited than these studies. The development of drug resistance in human pathogens against commonly used antibiotics has necessitated a search for new antimicrobial substances from other natural sources.

Marine sponges are known as chemical factories because they produce hundreds of unique chemical compounds that have been isolated and their structures have been determined [1]. They are known to produce a large number and diversity of secondary metabolites. Until now, more than 5000 different compounds have been isolated from about 500 species of sponges [2]. The new metabolites and its biological effects from sponges have been reported in scientific research papers in the last decades. The compounds exhibit a wide range of activities, including antibacterial, antifungal, antiviral, and antitumor activities. The isolated compounds also show a wide variation in chemical structure such as terpens, peptides, alkaloids and fatty acids [3]. Up to 800 antibiotics have been isolated from marine sponges [2].

The fact that our country is surrounded by seas on three sides which host a great diversity of sponge species is an invaluable biological wealth for Turkey. The effects of antimicrobial agents derived from the sponge on microorganisms, their chemical structures and mechanisms of action should be explored and made useful for humankind. Therefore, the antimicrobial activity of three different marine sponges collected from the Gulf of Saros, Turkey, against some bacteria and fungi was investigated in this study.

## MATERIALS AND METHODS

### Sampling and identification

Three different species of sponges were collected from the Gulf of Saros in the Aegean Sea in Turkey by Self Containt Underwater Breathing Aparatus (SCUBA), between 3 and 30 m depths. The sponge samples were placed inside sterile bags underwater and transferred to the laboratory using ice boxes.

Taxonomic identification of sponge samples was carried out by Prof. Dr. Melih Ertan Çınar (The Fisheries Faculty of Ege University, Department of Marine Biology- İzmir/Turkey) based on skeletal slides and dissociated spicule mounts. The sponge species investigated in this study are listed in Table 1.

**Table1. Sponge species investigated in this study**

| Diving Station                            | Diving Method | Depth   | Types of Sampled Sponge | Number of Samples |
|---|---------------|---------|-------------------------|-------------------|
| Station 1<br>The Gulf of Soros -Gallipoli | SCUBA         | 3-4 m   | Aplysina erophoba       | 6                 |
| Station 2<br>The Gulf of Soros -Gallipoli | SCUBA         | 10-30 m | Spongia agaricina       | 7                 |
| Station 3<br>The Gulf of Soros -Gallipoli | SCUBA         | 10 m    | Spongia officinalis     | 7                 |

### **Extraction**

A modified version of McCaffrey and Endean (1985) method was used for extraction. Dried sponges were cut into small pieces, removed from stones, shells and other remnants. The cut sponge pieces were kept in an oven at 105 ° C for 1h. Then, the pieces were turned into powder in aseptic conditions in a blender. These sponge samples were labeled in tiny glass jars [4].

Methanol-based organic solvent was chosen as the most efficient at extracting bioactive compounds from marine sponges [5]. Therefore, methanol was used as solvent in the extraction process.

Extraction process was carried out with soxhlet device in the central laboratory of Çanakkale Onsekiz Mart University (COMU). In extraction process, the sample rate of methanol was set to (1:10 v/v). Extraction of each sample was continued for about 24 hours. The extract was filtered using Whatman filter paper no 1. After the extraction process, methanol was blown with the aid of rotary evaporator at 55 ° C. The resulting extract was stored in labeled sterile screw capped bottles at -20°C.

### **Microbial strains**

In the study, *Bacillus cereus* ATCC 7064, *Bacillus subtilis* ATCC 6633, *Escherichia coli* ATCC 25902, *Staphylococcus aureus* ATCC 6538, *Staphylococcus epidermidis* ATCC 122228, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Aspergillus niger*, *Aspergillus fumigatus* var. *elipticus*, *Aspergillus flavus*, *Penicillium granulatum*, *Penicillium rugulosum*, *Penicillium jenseii*, *Aspergillus candidus*, *Geotrichum candidum* and *Candida albicans* ATCC 10239 were used as test microorganisms.

**Screening for antimicrobial activities:** In this study, the antimicrobial activity was determined using the disk diffusion method. For this, the extracts of the concentration of 25µL were impregnated to 6 mm diameter sterile paper disks with the automatic pipette. As a negative control, (methanol: distilled water, 04: 01) was prepared and 25µL of it was impregnated to the 6 mm diameter sterile paper disks [6]. Mueller-Hinton Agar was used for the determination of antimicrobial activity for bacteria and Potato Dextrose Agar for fungus strains.

On the other hand, Brain Heart Infusion Broth was used to activate bacterial cultures and Malt Extract Broth to activate fungus cultures. While bacteria were incubated at 35 ± 0.1 ° C for 24 h, fungus strains were incubated at 25 ° C ± 0.1 ° C for 72 h.

After incubation, the final inoculum concentration for bacteria and fungus strains was set to approximately 10<sup>6</sup> CFU / mL and 10<sup>8</sup> CFU / mL, respectively 0.1 mL of bacterial and fungal cultures was separately inoculated on mediums. Then the antibiotic discs and the discs injected with sponge extracts samples were placed on the plates. After the plates were kept at +4<sup>0</sup>C for 2 h, they were incubated at 35 ± 0.1 <sup>0</sup>C for 24 h for bacteria and at 25 ± 0.1 <sup>0</sup>C for 48h for fungus strains. At the end of the incubation period, the diameters of inhibition zones occurred on the mediums were evaluated in millimeters. In addition, the discs, which were impregnated with solvent, were compared to the standard antibiotic discs for control. The experiments were run in triplicate [7].

## **RESULTS AND DISCUSSION**

Antibacterial and antifungal activity of three different marine sponge's methanol extracts against seven bacterial and nine fungal test microorganisms are summarized in Table 2.

**Table2. Antimicrobial activity of three different marine sponge extracts and standard antibiotics against test microorganisms**

| Test Microorganisms                    | Inhibition Zones (mm)* |      |      |                         |       |         |         |    |
|--|------------------------|------|------|-------------------------|-------|---------|---------|----|
|  | Extract 25µL           |      |      | Comparative Antibiotics |       |         |         | NC |
|  | SE1                    | SE2  | SE3  | P 10                    | AM 25 | KETO 20 | NYS 100 |    |
| Bacillus cereus ATCC 7064              | 14.0                   | 10.0 | –    | 14.0                    | 12    | NT      | NT      | –  |
| Bacillus subtilis ATCC 6633            | 16.3                   | 9.30 | 7.0  | 13.0                    | 16    | NT      | NT      | –  |
| Staphylococcus epidermidis ATCC 122228 | 19.6                   | 13.6 | –    | 28.0                    | 25    | NT      | NT      | –  |
| Staphylococcus aureus ATCC 6538        | 17.3                   | 10.0 | 7.6  | 23.0                    | 20    | NT      | NT      | –  |
| Enterococcus faecalis                  | 14.3                   | –    | –    | 15.0                    | 13    | NT      | NT      | –  |
| Escherichia coli ATCC 25902            | 12.6                   | 7.30 | –    | 18.0                    | 19    | NT      | NT      | –  |
| Klebsiella pneumoniae                  | 14.3                   | 8.30 | –    | 14.0                    | 13    | NT      | NT      | –  |
| Aspergillus niger                      | –                      | 8.30 | –    | NT                      | NT    | 10.6    | 13.3    | –  |
| Aspergillus fumigatus var. elipticus   | –                      | 8.60 | –    | NT                      | NT    | 10.0    | 20.0    | –  |
| Aspergillus flavus                     | –                      | 19.6 | –    | NT                      | NT    | 21.3    | 20.0    | –  |
| Aspergillus candidus                   | –                      | 15.3 | 12.6 | NT                      | NT    | 14.0    | 15.3    | –  |
| Penicillium granulatum                 | –                      | 20.0 | –    | NT                      | NT    | 14.0    | 14.3    | –  |
| Penicillium rugulosum                  | 7.60                   | 11.0 | 11.6 | NT                      | NT    | 16.0    | 13.0    | –  |
| Penicillium jenseii                    | 9.30                   | 9.00 | –    | NT                      | NT    | 17.3    | 16.6    | –  |
| Candida albicans ATCC 10239            | 23.6                   | 20.6 | –    | NT                      | NT    | 21.0    | 20.0    | –  |
| Geotrichum candidum                    | 19.6                   | 12.6 | –    | NT                      | NT    | 23.3    | 16.6    | –  |

SE: Sponge Extracts

SE1: Antimicrobial activity of *Aplysina aerophoba*SE2: Antimicrobial activity of *Spongia agaricina*SE3: Antimicrobial activity of *Spongia officinalis*

P 10: Penicillin (10 µg) KETO 20: Ketocanazole (20 µg) AM 25: Ampicillin (25 µg),

NY 100: Nystatin (100 µg), (NT): Not Tested, NC: Negative Control

(–): No Inhibitions Zones

(\*): The figures show the diameters of inhibition zones. Each disc is 6 mm in diameter and impregnated with 25 µL (microliters) of extract.

The results proved that *Aplysina aerophoba*, *Spongia agaricina*, *Spongia officinalis* sponge extracts had antimicrobial activity, in different levels, against the test microorganisms in this study. The most intense antimicrobial activity against the Gram-positive and the Gram-negative test bacteria was obtained from the extracts of the sponge *Aplysina aerophoba*. However, it was found out that the extracts from *Spongia agaricina* had the most intense antimicrobial activity against the test fungi. On the other hand, *Spongia officinalis* extract did not affect the test microorganisms except two bacteria and moulds.

In the study, *Aplysina aerophoba* sponge extract showed a high antimicrobial activity against Gram-positive bacteria *Bacillus cereus* ATCC 7064 and *Bacillus subtilis* ATCC 6633, *Staphylococcus epidermidis* ATCC 122228 and *Staphylococcus aureus* ATCC 6538. It displays 14.0 mm, 16.3mm, 19.6 mm and 17.3 mm inhibition zone against these bacteria, respectively. At the same time, *Aplysina aerophoba* sponge extracts showed 14.3mm, 12.6mm and 14.3mm-inhibition zone against a Gram negative bacteria *Enterococcus faecalis*, *Escherichia coli* and *Klebsiella pneumoniae*, respectively. In addition, the extract of *Aplysina aerophoba* developed a 14.3 mm and 16.3mm inhibition zone against Gram negative bacteria *Klebsiella pneumoniae* and Gram- positive bacteria *Bacillus subtilis*, so they had higher antimicrobial activity than Penicillin and Ampicillin.

On the other hand, the extract of *Aplysina aerophoba* formed a 23.6 mm inhibition zone, against *Candida albicans* ATCC 10239, in particular. Therefore, it was determined that it had higher antifungal activity than Ketoconazole and Nystatin, which were used in the study.

*Spongia agaricina* extract exhibit more antimicrobial activity than Ketoconazole and Nystatin with 20 mm-inhibition zone against *Penicillium granulatum*

Amade *et al.* (1987) reported that the sponge *Aplysina cavernicola* collected from the Mediterranean had a weak antimicrobial activity against *Staphylococcus aureus* and *Bacillus subtilis*. In addition *Spongia officinalis* sponges did not show any inhibition zone against the bacteria *Bacillus subtilis*, *Staphylococcus epidermidis*, *Escherichia coli* or against the fungi *Aspergillus niger* and *Aspergillus fumigatus* or the yeast *Candida albicans* [8].

Hentschel *et al.* (2001) reported that *Aplysina aerophoba* and *Aplysina cavernicola* from Mediterranean sponges exhibited antimicrobial activities against Gram- positive and Gram negative reference strains but not against the fungus *Candida albicans*. [9].

Mccaffrey and Endean R., (1985) assessed the antimicrobial activity against test bacteria and fungi activities of extracts from species of sponge collected from Queensland, Australia. They found that antimicrobial activity exhibited by sponges is more prevalent in temperate than in tropical species [5].

Different antimicrobial activity results were obtained by researchers for similar sponges from the different country seas. In recent years, interest in natural products such as marine sponges has dramatically increased because infectious diseases represent important human health problem. New products can be developed from potential marine compounds. Considerable efforts should be made for the characterization of secondary metabolites to develop new medicines from marine sources with a multidisciplinary effort [10, 11, 12]. We have shown three different marine sponges extracts test bacteria and fungi. In further research, new drugs against many infections and diseases can be obtained from these marine sponges.

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