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# Antimicrobial Activity of Cyanobacteria Isolated From Hot Spring of Geno

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**Abstract:** Cyanobacteria (blue-green algae) are rich sources of structurally novel and biologically active metabolites. Recent studies indicate the presence of some bioactive compounds in the blue green algae which are shown to exhibit anticancer, antimicrobial, antifungal or anti-inflammatory and other pharmacological activities. The present study aimed to collect and identify cyanobacteria from hot spring of Geno in Bandar Abbas province. Totally 21 species of cyanobacteria were collected and cultured in BG-11 medium. Based on their growth characteristics; seven species namely *Oscillatoria subbrevis, O. tenuis, O. limentica, O. angusta, O. articulate, Synechocystis aquatilis* and *Synechoccous cerdorum* were selected for the production of antimicrobial agents against five Gram-positive (*Bacillus subtilis, B. pumulis, Enterococcus fecalis, Staphylococcus aureus* and *S.epidermidis*) and three Gram-negative (*Escherichia coli, Pseudomonas aeruginosa* and *Klebsiella pneumoniae*) bacteria and two fungi (*Candida albicans* and *Saccharomyces cerevisiae*). The results of this study showed that the methanol extracts exhibited high antimicrobial activity against some Gram positive bacteria (*Bacillus subtilis* and *B. pumulis*), moderate antibacterial activity against the some Gram negative organisms (*Escherichia coli*) and moderate antifungal activity against some fungi (*S. cerevisiae*).

**Key words:** Antimicrobial · *Cyanobacteria* · Hot Spring · Geno · Metanol Extracts

## INTRODUCTION

Cyanobacteria, the blue green algae are one of the most diverse groups of Gram-negative photosynthetic prokaryotes widely distributed throughout the world. Cyanobacteria are known to produce a wide range of secondary metabolites with various biological actions [1]. Recent studies indicate the presence of some bioactive compounds in the freshwater blue green algae which are shown to exhibit anticancer, antimicrobial, antifungal or anti-inflammatory and other pharmacological activities [2].

The screening of extracts or isolated compounds from different natural sources is a common way to discover biological active metabolites. Research into bioactive metabolites includes studies to discover a new antibiotic or cytotoxic metabolite of microalgae as well as to discover cyanobacteria which are toxic to other cyanobacteria or green algae. In such research activities, cyanobacteria were found to be a rich source for various products of commercial, pharmaceutical or toxicological interest [3].

Because of the increasing resistance to antibiotics of many bacteria, plant compounds and other microorganisms are of new interest as antiseptics and antimicrobial [4]. The aim of this study was to test extracts from various cyanobacteria isolated from Geno hot spring, Bandar Abbas (Iran) against a diverse range of organisms comprising Gram-positive and Gram-negative bacteria and yeasts to evaluate its antibacterial and antifungal activities.

## MATERIALS AND METHODS

**Cyanobacteria Samples** Water samples containing cyanobacteria were collected from various sites of Geno hot spring, Bandar Abbas, Iran, during different seasons in 2011. Samples were cultured directly in BG-11 media [5], after colonization, cyanobacteria were transferred to the same medium [6] and the subculturing method was used to prepare unialgal growth [7]. Identification was carried out using morphological variation and taxonomical approaches according to Desikachary [8] and Prescott [9].

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**Preparation of Algal Extracts:** Ten days old algal culture was centrifuged; pellet was collected, weighted and used for antimicrobial activity. 0.5 g of each of algae pellets was extracted in 10 ml methanol. All the extracts were preserved at 4°C.

**Microbial Strains:** The antimicrobial activity of Cyanobacteria extracts isolated from hot spring were individually tested against a panel of microorganisms, including; *Bacillus subtilis* (ATCC 465), *Enterococcus fecalis* (ATCC 29737), *Staphylococcus aureus* (ATCC 25923), *Staphylococcus epidermidis* (ATCC 12228), *Escherichia coli* (ATCC 25922), *Klebsiella pneumoniae* (ATCC 10031), *Pseudomonas aeruginosa* (ATCC 85327), *Candida albicans* (ATCC 10231) and *Saccharomyces cerevisiae* (ATCC 9763). Bacterial strains were cultured overnight at 37°C in Mueller Hinton agar (MHA). Yeast was cultured overnight at 30°C in Sabouraud's dextrose agar (SDA).

**Antimicrobial Screening by the Disk Diffusion Method:** The disc diffusion method was applied for the determination of antimicrobial activity of the prepared extracts [10]. Extracts were dissolved in dimethyl sulfoxide (DMSO). A suspension of the tested microorganism (0.1 ml of $10^8$ cells/ml) was spread over the surface of agar plates (MHA and SDA). Filter papers having a diameter of 6 mm, soaked with 25 µL of each extracts were placed on the inoculated agar plates. Before incubation, all Petri dishes were kept in the refrigerator (4°C) for 2 h. then they were incubated at 37°C for 24 h for bacteria and at 30°C for 48 h for the yeast. The diameters of the inhibition zones were measured in millimeters.

**Minimum Inhibitory Concentrations (MIC):** MIC values were determined by broth microdilution assay recommended by the NCCLS [11]. Serial two-fold dilutions of the extracts were made in Mueller-Hinton Broth containing 0.5% Tween 80 for bacteria and Sabouraud's Dextrose Broth with 0.5% Tween 80 for fungi in 96-well microtiter plates. Fresh microbial suspensions prepared from overnight grown cultures in the same media were added to give a final concentration of $5 \times 10^5$ organisms/ml. Controls of medium with microorganisms or the extract alone were included. The plates were incubated at 37°C for 24 h for bacteria and 30°C for 48 h for fungi. The first dilution with no microbial growth was recorded as MIC.

**Statistical Analysis:** The statistical analysis was performed with statgraphics (Centurion XV) and Excel software. The multi-factorial ANOVA analysis was followed by the Tukey multiple comparison tests for statistical comparisons. P-value of less than 0.05 was assumed for significant differences.

## RESULTS AND DISCUSSION

Totally 21 species of cyanobacteria were collected from the hot spring and cultured in BG-11 medium. Based on their growth characteristics; seven species namely *Oscillatoria subbrevis, O. tenuis, O. limentica, O. angusta, O. articulate, Synechocystis aquatilis* and *Synechoccous cerdorum* were selected for antimicrobial activity.

Data on antimicrobial activity in terms of inhibition zones exhibited by the cyanobacteria methanol extract were shown in Tables 1 and 2. Result showed that all Cyanobacteria extracts were found to have high activity against *B. subtilis*, *S. epidermidis* and *B. pumullis*. *P. aeruginosa* and *K.pneumoniae* were resistant to all tested algal extracts.

All extracts inhibited slightly the growth of *E. coli, S. aureus* and *E. fecalis* (Table 1).

Results obtained from disc diffusion method, followed by measurements of MIC, indicated that *S.epidermidis* was the most sensitive among tested organisms, since the methanol extract of cyanobacteria showed lowest MIC value (3.75 mg/ml) (Table 2 ).

The susceptibility varied with the test bacteria. In general, Gram positive bacteria seemed to be more sensitive to the cyanobacterial extracts than Gram negative bacteria. The extracts with the antibacterial action were also active on fungi. However, treatment must be continued over a longer period. The standard drugs used namely, nystatin, tetracycline and gentamicin exhibited antimicrobial activities nearly the same as the Cyanobacteria extracts against some of the tested organisms. None of the Cyanobacteria methanol extract showed antifungal activity against *Candida albicans*, except for *Synechocystis aquatilis.*

Many investigators mentioned that the extract of Cyanobacteria revealedantibacterial activity against *B. subtilis*, *S. epidermidis*, *B. pumullis* and *P. aeruginosa* [12, 13]. Also, the methanolic extract of cyanobacteria showed antimicrobial activity against *Proteus vulgaris*, *Bacillus cereus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *Aspergillus flavus*

Table 1: Antimicrobial activity of cyanobacteria methanol extracts by disc diffusion method

| | Cyanobacteria species extracts (IZ in mm) | | | | | | | Antibiotic (IZ in mm) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Microorganisms | OS.SUB | OS.TEN | OS.LIM | OS.ANG | OS.ARTI | SYN.AQU | AYN.CER | Tet | Gen | Nys |
| *B. subtilis* | 20±0.7 | 18±0.7 | 19±0.6 | 16±0.9 | 19±0.7 | 22±0.6 | 20±0.9 | 21±0.7 | 0 | Nt |
| *B.pumullis* | 22±0.5 | 20±0.5 | 18±0.9 | 17±0.6 | 20±0.5 | 20±0.5 | 19±0.6 | | | |
| *E. faecalis* | 14±0.9 | 10±0.7 | 11±0.5 | 0 | 15±0.9 | 16±0.7 | 14±0.9 | 9±0.9 | 0 | Nt |
| *S. aureus* | 16±0.6 | 12±0.4 | 14±0.7 | 12±0.4 | 15±0.6 | 17±0.5 | 15±0.4 | 20±0.6 | 0 | Nt |
| S. epidermidis | 24±0.7 | 20±0.6 | 17±0.5 | 14±0.7 | 19±0.5 | 23±0.5 | 22±0.5 | 34±0.5 | 0 | Nt |
| *E. coli* | 15 | 11±0.9 | 14±0.4 | 10 | 14±0.7 | 17±0.4 | 15 | 0 | 23±0.4 | Nt |
| *P. aeruginosa* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 12±0.6 | Nt |
| *K. pneumoniae* | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 20±0.9 | Nt |
| *C. albicans* | 0 | 0 | 0 | 0 | 0 | 12±0.3 | 0 | Nt | Nt | 18±0.7 |
| *S. cerevisiae* | 12±0.7 | 0 | 10±0.5 | 0 | 12±0.4 | 16±0.9 | 14±0.6 | Nt | Nt | 18±0.9 |

Values given as mean of triplicate tests. IZ: Inhibition zone ( 20 µl per disc). OS.SUB (*Oscillatoria subbrevis*),O S. TEN (*O. tenuis*),OS.LIM (*O. limentica*), OS.ANG (*O. angusta*), OS. ARTI (*O. articulate*), SYN. AQU (*Synechocystis aquatilis*), SYN. CER (*Synechoccous cerdorum*). Tet: Tetracycline, tested at 30 µg/disc. Gen: Gentamicin, tested at 10 µg/disc. Nys: nystatin, tested at 30 µg/disc. Nt = not tested

Table 2: MIC values of methanol extracts of Cyanobacteira isolated from Geno hot spring

| | Cyanobacteria species | | | | | | | Antibiotic (IZ) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| Microorganisms | OS.SUB | OS.TEN | OS.LIM | OS.ANG | OS.ARTI | SYN.AQU | AYN.CER | Tet | Gen | Nys |
| *B. subtilis* | 3.75±0.7 | 3.75±0.5 | 3.75±0.4 | 7.5±0.7 | 3.75±0.8 | 3.75±0.8 | 3.75±0.7 | 3.2±0.7 | 0 | Nt |
| *B.pumullis* | 3.75±0.5 | 3.75±0.8 | 3.75±0.7 | 7.5±0.2 | 3.75±0.5 | 3.75±0.7 | 3.75±0.5 | | | |
| *E. fecalis* | 15±0.8 | 15±0.2 | 15±0.8 | - | 7.5±0.7 | 7.5±0.2 | 7.5±0.8 | 6.4±0.5 | 0 | Nt |
| *S. aureus* | 7.5±0.2 | 7.5±0.4 | 7.5±0.5 | 15±0.5 | 7.5±0.2 | 3.75±0.5 | 7.5±0.7 | 3.2±0.5 | 0 | Nt |
| S. epidermidi | 3.75±0.7 | 3.75±0.8 | 3.75±0.2 | 7.5±0.7 | 3.7±0.5 | 3.75±0.4 | 3.75±0.2 | 1.6±0.8 | 0 | Nt |
| *E. coli* | 7.5±0.8 | 15±0.5 | 15±0.7 | >15±0.8 | 15±0.4 | 7.5±0.7 | 3.75±0.5 | 0 | 3.2±0.2 | Nt |
| *P. aeruginosa* | - | - | - | - | - | - | - | 0 | 3.2±0.9 | Nt |
| *K. pneumoniae* | - | - | - | - | - | - | - | 0 | 6.4±0.5 | Nt |
| *C. albicans* | - | - | - | - | - | 15±0.8 | - | Nt | Nt | 3.2±0.8 |
| *S. cerevisiae* | 15±0.7 | - | >15±0.8 | - | 15±0.7 | 7.5±0.7 | 7.5±0.8 | Nt | Nt | 1.6±0.7 |

Valeus given as mean of triplicate tests. MIC: Minimum inhibitory concentration values in mg/ml. OS.SUB (*Oscillatoria subbrevis*), OS. TEN (*O. tenuis*),OS.LIM (*O. limentica*), OS.ANG (*O. angusta*), OS. ARTI (*O. articulate*), SYN. AQU (*Synechocystis aquatilis*), SYN. CER (*Synechoccous cerdorum*). Tet: Tetracycline, tested at 30 µg/disc. Gen: Gentamicin, tested at 10 µg/disc. Nys: nystatin, tested at 30 µg/disc. Nt = not tested

[14]. Unicellular marine cyanobacteria such as Synechocystis and Synechococcus reportedly cause inhibition of Gram positive bacteria [15]. The antimicrobial activity of the extract could be due to the present of different chemicals that may include fatty acids, alkaloids, flavinoides and phenolics compound [7, 16, 17].

It can be concluded from this study that the extract obtained from some Cyanobacteria strains isolated from the hot spring showed antimicrobial activity against the pathogens used in the present investigation. Further researches should be made to identify and purify natural products from these Cyanobacteria with antimicrobial activity.

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