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Antibacterial and Antifungal Activities of Cyanobacterial Strains Isolated from Hot Springs in Oman

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ABSTRACT: In this study, cyanobacterial microbial mats from five hot springs in Oman, namely Al Kasfah Rustaq, Al Thwara Nakhl, Al–Ali Hammam, Gala and Bowsher, were characterized using direct microscopy. Nine monoclonal cyanobacterial cultures were obtained and their extracts in butanol, dichloromethane (DCM) and hexane were screened for antibacterial and antifungal activities. Direct microscopy revealed the presence of 12 different unicellular and filamentous morphotypes, with different distribution in the various mats. Temperature seems to be one of the most important parameters that accounts for the differences in cyanobacterial composition of the mats. Cells of the nine isolates and their aqueous supernatants were subsequently extracted with butanol, DCM and hexane. Dried extracts were tested against nine bacterial (i.e. gram +ve *Staphylococcus aureus*, *Bacillus subtilis* and gram –ve, *Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella choleraesuis, S. enterica*, *Psuedomonas aeruginosa*, *Providencia stuartii*, and *Acinetobacter calcoaceticus*) and two fungal pathogens (*Rhizoctonia solani* and *Pythium* sp.). All isolates exhibited antibacterial and antifungal activities, which depended mainly on the type of cyanobacterial culture, type of solvent used and the pathogen tested. The highest antibacterial activity was observed in *Phormidium* species, and butanol was found to be the most appropriate solvent to extract bioactivity from these cyanobacterial species. The results of this study suggest that thermal springs in Oman harbor diverse types of cyanobacteria, which may constitute an important source of antibacterial and antifungal compounds. Further investigation is needed to purify these compounds and find their chemical compositions and modes of action.

Keywords: Cyanobacterial mat; Antibacterial; Antifungal.

فاعلية المضادات البكتيرية و الفطرية التي تفرزها السيانوبكتيريا المعزولة من الينابيع الساخنة في عمان

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ملخص: في هذه الدراسة تم فحص التجمعات السيانوبكتيرية البكتيرية في خمس ينابيع ساخنة في عمان وهى عين الكسفة في الرستاق و الثوارة في نخل و الحمام و غلا وبوشر باستخدام المجهر. من هذه التجمعات تم الحصول على تسع سلالات من السيانوبكتيريا و الكشف عن قدرتها على أفراز مركبات لقتل البكتيريا و الفطريات. باستخدام المجهر تم الكشف عن 12 نوع مختلف من السيانوبكتيريا وحيدة الخلية و خيطية موزعة بشكل مختلف في هذه التجمعات. كما تبين أن درجة الحرارة هي المسؤولة عن هذه الاختلافات .وقد تم الحصول على مستخلص من هذه السلالات باستخدام الماء و البيوتانول و الداي كلوروميثين و الهيكسين .وقد جربت المستخلصات الجافة ضد تسع أنواع من البكتيريا و نوعين من الفطريات و هم : *Staphylococcus aureus*, *Bacillus subtilis* and gram –ve, *Escherihia coli*, *Klebsiella pneumoniae*, *Salmonella choleraesuis, S. enterica*, *Psuedomonas aeruginosa*, *Providencia stuartii*, and *Acinetobacter calcoaceticus*) (*Rhizoctonia solani* and *Pythium* sp.) .جميع هذه السلالات كان لها القدرة على إبادة البكتيريا و الفطريات حسب نوع السيانوبكتيريا و نوع البكتيريا الضارة ونوع المذيبات .نتائج هذه الدراسة تقترح أن الينابيع الساخنة في عمان تحتوي على سلالات من السيانوبكتيريا لها القدرة على إبادة الفطريات و البكتيريا الضارة.و في المستقبل يجب القيام بأبحاث مفصلة لتنقية هذه المركبات و الكشف عن تركيبها الكيميائي و طريقة فعاليتها.

كلمات مفتاحية: السيانوبكتيريا، مضادات البكتيريا، مضادات الفطريات ،التجمعات البكتيرية والينابيع الساخنة و عمان.

1. Introduction

Terrestrial geothermal springs are characterized by a dominance of microbial mats comprising a diversity of prokaryotic organisms, principally cyanobacteria [1-4]. The biodiversity of thermophilic cyanobacteria in

geothermal springs has long attracted the attention of microbial ecologists because of their unique adaptations to these harsh environments [3, 5-8]. However, their value as a source of thermo-stable bio-compounds and unusual metabolic products for biotechnological applications has received much less attention [9, 10]. In recent decades, the search for bioactive compounds has been more directed towards the exploration of new environments and the screening of less exploited microbial groups endowed with a more versatile secondary metabolism such as cyanobacteria [11]. Therefore, the exploration of bioactive compounds from thermophilic and thermotolerant cyanobacteria of hot springs is an important step towards these goals.

Cyanobacteria produce a wide variety of compounds including 40% lipopeptides, 5.6% amino acids, 4.2% fatty acids, 4.2% macrolides and 9% amides [12]. Lipopeptides are biochemically active, having cytotoxic, anticancer, antibiotic, enzyme inhibitor, antiviral and antifungal activities [11]. Numerous screening programs have revealed the potential of cyanobacteria in the production of novel antimicrobial [13-19], antifungal [20-22], antiviral [23], anticancer or cytotoxic [24] and antifouling compounds [10]. Cyanotoxins like the hepatotoxic microcystins and nodularins or the neurotoxic anatoxins and saxitoxins have been isolated from cyanobacteria [25, 26].

Thermotolerant cyanobacteria have also been reported to produce bioactive compounds [9,11]. The thermotolerant *Phormidium* sp. has produced an antimicrobial compound against *Candida albicans* and *Cladosporidium resinae* [27]. Anticancer drugs have also been made from thermophilic cyanobacteria [28]. The thermophilic *Synechococcus* sp. is a potential producer of poly-hydroxybutyrate, which is the basis of biologically degradable plastics [29].

In this study, we identified five locations in northern Oman with active thermal springs, dominated by cyanobacteria mats [9]. It was previously demonstrated that microbial mats from these springs had antimicrobial activity *in vivo* [9]. The aim of this study was to identify the common mat-forming cyanobacteria in these hot springs using direct microscopy, and to isolate cyanobacterial cultures and investigate their antibacterial and antifungal activities.

2. Material and methods

2.1 Physical and chemical characteristics of the sampling sites

Mat samples were collected from Al-Kasfah spring Rustaq, Al-Thwara Nakhl spring, Al-Ali Hammam spring, and Gala and Bowsher springs in sterile plastic boxes. Al-Ali Hammam spring, located in the huge fault structure separating Triassic dolomite from ophiolite, had the highest temperature range recorded (60-67 ^{o}C), while Gala and Bowsher had the lowest temperatures (Table 1). Air-shade and water temperatures were taken in the field using an electronic thermometer (Spring Celsimeter, model super-K). The pH of the samples was measured by a digital pH meter (Orion; Thermo Fisher Scientific, Waltham, MA, USA) and electrical conductivity was determined using a conductivity bridge (Systronics, Norcross, GA, USA). Ammonium concentration [NH_4^+] was measured using a multi-parameter NH_4^+ electrode (Orion) as described in [30].

Table 1. Environmental conditions from the five investigated hot springs.

	Hammam	Rustaq	Nakhal	Boshwer	Ghala
Lat/Long coordinates	23^{o}28’10.5"N 58^{o}19' 13"E	23^{o}23’27"N 57^{o}25' 28"E	23^{o}23’N 57^{o}49'E	23^{o}55'76"N 58^{o}40' 96"E	23^{o}55'05"N 58^{o}38'33"E
Temperature (^{o}C)	60-67	46-47	37-40	35-38	35-37
pH	6.98	6.93	7.9	7.1	7.1
Conductivity ($\Omega^{-1}cm^2\,mol^{-1}$)	1900	1300	800	1100	1200
N (mg/L)	0.2	1.1	1.4	0.6	0.6
NO_3 (mg/L)	1	4.8	6	2.5	3

2.2 Isolation and identification of cyanobacteria

Cyanobacterial species were observed under the microscope within 24 hours of collection. Identification was performed using morphological variation studies and taxonomical approaches [31-34]. Cyanobacterial communities at different hot springs were compared using cluster analysis of the Bray-Curtis similarity index [35] with the help of PRIMER software (Plymouth Marine Laboratory). For construction of a similarity matrix, the presence of particular morphotypes was denoted as 1 and their absence was set as 0, and the data were square root transformed. Fourteen cyanobacteria species belonging to twelve cyanobacterial genera were identified . Monoclonal cultures were prepared using subculturing methods in BG11 medium (ATCC medium 616) with and without nitrate **[**34]. The BG 11 medium was prepared by adding $NaNO_3$ (1.5 g), K_2HPO_4 (0.04 g), $MgSO_4{\cdot}7H_2O$ (0.075 g), $CaCl_2{\cdot}2H_2O$ (0.036 g), ferric ammonium citrate (0.006 g), EDTA (disodium salt) (0.001 g), Na_2CO_3 (0.02 g) and trace metal mix A5 (1.0 ml) per

litre distilled water. Each isolated cyanobacterium was cultured in a 500 ml flask containing 250 ml of BG11 medium for 30 days. The cultures were incubated at 30-35 ^{o}C and illuminated for 16 hrs. Biomass was harvested at the end of the linear growth phase, filtered on nylon net and carefully washed with sterile BG11 media. These cultures were used for further investigations.

2.3 Preparation of cell extracts

The cultures were harvested after 30 days of growth by centrifugation at 5000 rpm for 15 minutes. Extracts of the cyanobacterial pellets were prepared, in each of 100 ml butanol, 100 ml DCM and 100 ml hexane. These extracts were dried under reduced pressure at 40 °C and were stored at −10 °C for further study.

2.4 Determination of antibacterial and antifungal activities

The extracts from the isolates were screened for antibacterial activity on nine different pathogenic bacterial strains and for antifungal activities on two fungal strains. Four of the pathogenic strains used were American Type Culture Collection (ATCC) of bacteria: *Escherichia coli* (ATCC 9637), *Klebsiella pneumoniae* (ATCC 37853), *Staphylococcus aureus* (ATCC 29213) and *Salmonella choleraesuis* (ATCC 14028), whereas *Salmonella enterica*, *Pseudomonas aeruginosa*, *Providencia stuartii*, *Acinetobacter calcoaceticus*, *Bacillus subtilis* were obtained from the Sultan Qaboos University Hospital (kind contribution from Dr. Akbar Rafay). The fungal plant pathogens of *Rhizoctonia solani* and *Pythium* sp. were obtained from Prof. Mike Deadman, SQU. The bacteria were subcultured on nutrient agar (Oxoid, Hampshire, England) at 37 °C for 24 hrs and the fungi were grown on potato dextrose agar (PDA; Oxoid, Hampshire, England) at 25 °C for 5 days.

The dried extracts were dissolved in their extraction solvent (final concentration = 1 mg/ml), and antibacterial and antifungal activities were determined by the disc diffusion method. Filter paper discs (6.4 mm) were saturated with 100 µl of the test solution, dried under laminar air flow, and placed on either the nutrient agar plate for bacteria or the potato dextrose agar plate for fungi, both of which had been inoculated with a lawn of the test microorganisms. Plates were incubated at 37 °C for a period of 24 hrs for bacteria, and at 25 °C for 5 days for fungi. Discs treated only with 100 µl butanol, or hexane or DCM, were used as negative controls.

The extracts containing antibacterial and antifungal components produced distinct, clear, circular zones of inhibition around the discs, and the diameters of the clear zones were determined and used as an indication of anti-bacterial and anti-fungal activity. The experiment was run three times in triplicate and the mean inhibition zones were calculated for each extract/treatment.

3. Results and discussion

The pH of the samples was between 7 and 7.9 and electrical conductivity was between 800-1900 µS/cm in all samples (Table 1). Ammonium concentrations were between 1 and 6 mg/l, with the highest value in Nakhl spring and lowest in Hammam spring.

Direct microscopy examinations revealed the presence of 14 unicellular and filamentous cyanobacterial morphotypes, in the microbial mats of the studied geothermal springs. Morphotypes like *Synechococcus*, *Aphanocapsa*, *Anabeana*, *Gloeocapsa*, *Chroococcus*, *Aphanizomenon*, *Leptolyngbya*, *Oscillatoria*, *Phormidium*, *Spirulina*, *Lyngbya* and *Nostoc* (Table 1) were observed. The highest number of cyanobacterial morphotypes (7) was recorded for Hammam and Bowsher hot springs.

Cluster analysis demonstrated that the cyanobacterial communities present in Nakhl and Gala were similar to each other, while those found in Rustaq thermal spring were completely different (Figure 1). Temperature differences in the mats were found to be one of the most important parameters that accounted for the differences in cyanobacterial composition (Table 1), with *Synechoccus and Aphanocapsa* observed in Hammam spring (60- 67 ^{o}C) and filamentous genera of *Phormidium*, *leptolyngbya* and *lyngbya* constituting most of the cyanobacterial mats in Bowsher (35-38 ^{o}C), Gala (37- 40 ^{o}C) and Nakhl (37-40 ^{o}C). Previous studies have also revealed the presence of unicellular forms like *Synechococcus* in cyanobacterial mats at thermal gradients from 50 ^{o}C to 75 ^{o}C [36,37], whereas those mats occurring at the lower end of thermophily (35-50 °C) are often dominated by filamentous cyanobacteria such as *Phormidium*, *Lyngbya*, *Oscillatoria*, *Pseudoanabaena*, *Calothrix* and *Fischerella* [3,37,38].

On the other hand, nitrogen also affects the species distribution in cyanobacterial mat communities below ~60 °C [3,38]. Diazotrophic cyanobacteria are able to colonize springs where nitrogen levels are too low to support other taxa, but they may be outcompeted by non-diazotrophic cyanobacteria in springs with sufficient combined nitrogen [38]. Our results also support this assumption since heterocystous species were not detected in Nakhl spring, where the ammonium level was the highest (Table 1), while heterocystous species like *Nostoc* sp. were observed in Bowsher spring which has a comparatively low nitrogen concentration.

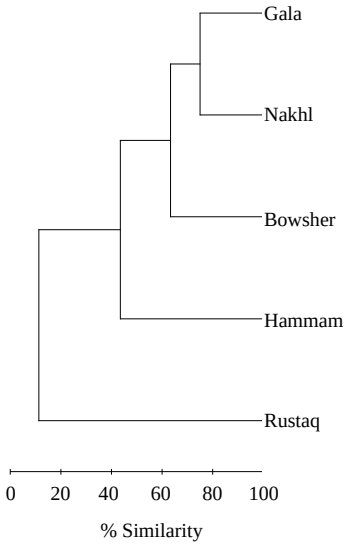

Figure 1. Cluster analysis of the presence and absence of cyanobacterial morphotypes in the studied hot springs, based on Bray-Curtis similarity matrix. Similarity between communities is in %.

Nine different monoclonal cyanobacterial isolates were obtained from all the mats (Figure 2). Based on their morphological features, the cultured strains were identified as species of genera *Phormidium, Chroococcus, Anabeana, Gloeocapsa, Lyngbya, Leptolyngbya* and *Nostoc* (Figure 2). All these strains were endowed with antibacterial activity (Table 2). The diameter of the inhibition zone depended mainly on the type of cyanobacterial species, type of solvent used and the pathogen tested. Almost all the tested extracts of cyanobacteria were highly effective against *E.coli* and *S. aureus*, but few extracts inhibited growth of *A. calcoaceticus* and *B. subtilis* (Table 2). Butanol was found to be the most appropriate solvent to obtain bioactive extracts from cyanobacterial biomasses. This solvent allows the extraction of relatively polar bioactive compounds. Extracts prepared in hexane (which extracts highly non-polar compounds) did not show bactericidal activity against any of the tested microorganisms except for *E. coli* (Table 2).This observation was in agreement with other studies [39], which state that hexane extracts show little antibacterial activity, and this could be due to the chemical nature of the active compounds [20].

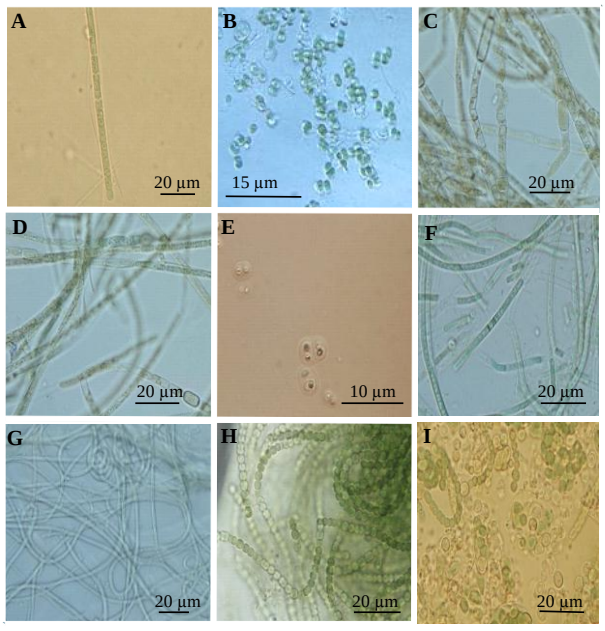

Figure 2. Photomicrographs of cyanobacterial isolates cultured from the microbial mats of the active thermal springs of Northern Oman. The identification of these morphotypes can be found in Table 2 with the same letter code indicated in brackets.

Table 2. Cyanobacterial morphotypes observed from the five investigated hot springs.

	Hammam	Rustaq	Nakhl	Bowsher	Gala
Cyanobacterial morphotypes					
Synechoccus	+	-	-	-	-
Aphanocapsa	+	-	-	+	-
Aphanothece	+	-	-	+	-
Chroococcus	-	-	+	+	-
Gleocapsa	-	-	+	+	+
Aphanizomenon	-	+	+	-	-
Lyngbya	+	-	-	-	-
Leptolyngbya	+	-	+	+	+
Oscillatoria	-	+	-	-	-
Phormidium	+	-	+	+	+
Spirulina	+	+	-	-	-
Nostoc	-	-	-	+	-
Total number	7	3	5	7	3

+ present; - absent

Among butanol extracts, the highest antibacterial activity was detected in *Phormidium* species, which had the ability to inhibit the growth of all tested pathogens (Table 2). The largest inhibition zone for butanol extracts of *Phormidium* species was observed in the case of Gram-negative *P. stuartii*, *S. enterica* and *P. aeruginosa* respectively (Table 2). The second highest inhibition zone was detected in the case of *K. pneumoniae*, *B. subtilis* and *E.coli*. *Phormidium* spp. have been previously reported to produce compounds that inhibit growth of different Gram-positive and Gram-negative bacterial strains, yeasts, and fungi [40-42]. Butanol extracts of *Lyngbya* sp. exhibited the highest antibacterial activity against *K. pneumoniae* (Figure 3). *Lyngbya* species are known for the production of antimicrobial, anticancer and quorum sensing inhibitory compounds [10]. Significant antimicrobial activity was observed by using *Chroococcus* sp. against all pathogens. These results are congruent with previous reports that detected high biological activity of cyanobacterial species belonging to the genera of *Phormidium*, *Chroococcus*, *Lyngbya* and *Leptolyngbya* [22,38,43-45]. The butanolic extracts of *Nostoc* sp.1 and *Nostoc* sp. 2 strains strongly inhibited the growth of *S. choleraesuis*, *P. stuartii* and *P. aeruginosa* (Table 2). Studies on the Antarctic cyanobacterium *Nostoc* CCC537 have shown that methanolic extracts of this strain inhibit the growth of *Mycobacterium tuberculosis*, *Enterobacter aerogenes*, *Salmonella typhi*, *E. coli*, *P. aeruginosa* and *S. aureas* [46]. An intracellular biomolecule similar to an anthraquinone (indane) derivative of a diterpenoid was hypothesized to be the active compound. Some of the active substances screened from *Nostoc* include Nostocyclyne, Nostofungicidine, Nostocin [47,48], 4,4'-dihydroxybiphenyl [21], cryptophycin-A and Nostodione-A, which inhibit microtubule assembly or function [49].

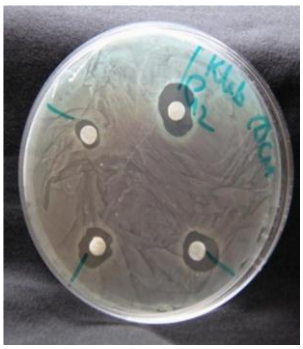

Figure 3. Inhibition of *Klebsiella pneumoniae* growth by DCM extracts of *Nostoc*, *Leptolyngbya*, *Anabeana* species.

In the present study, we observed that the different isolates of the genus *Anabeana* exhibited different antimicrobial activities. Extracts of *Anabeana* sp.1 were more effective against *E.coli* and *K. pneumonia* compared to *Anabeana* sp. 2 (Table 2). It has been shown hat different species of cyanobacteria produce extracellular compounds

with different antimicrobial activities, such as amino acids, peptides, lipopeptides, amides, fatty acids, indoles, nucleosides, alkaloids, polyketides and sterols [50,51]. *Calothrix brevissima* and *Oscillatoria redekei* have antibacterial activity and produce bromophenols [52] and fatty acids [51], respectively. Two depsipeptide bioactive compounds, scyptolin A and B have been isolated from the axenically grown terrestrial cyanobacterium *Scytonema hofmanni* [53]. The cyanobacterial antibacterial compounds inhibit the growth of Gram-positive pathogens by destabilizing their cytoplasmic membrane [54]. In the Gram-negative bacteria, the outer membrane of the bacteria shows the hydrophilic surface on the side chains of lipopoly-saccharides, which prevents the entrance of hydrophobic substances to the cellular membrane [55]. However, certain molecules, such as terpenoid and phenolic compounds, can destabilize the lipopolysaccharide layer [56]. Bioactive compounds have not been isolated through this study and the isolation of antibacterial compounds from thermal spring cyanobacteria should be a priority in future investigations.

All cyanobacterial isolates showed antifungal activity against *Rhizoctonia solani* and *Phythium* species (Table 3).

Table 3. Inhibition of growth of bacterial pathogens by buthanol, dichloromethane (DCM), and hexane extracts of cyanobacterial isolates. Data are the mean diameters ± standard deviation (SD) of inhibition zones (mm). Cyanobacterial buthanol, DCM and hexane extracts were applied to sterile paper disks (diameter = 6.4 mm). Inhibition zones in the presence of the positive control ie. Streptomycin antibiotic was between 15-32 mm.

Cyanobacterial spp.	Solvent	*S. aureus*	*E. coli*	*K. pneumonia*	*S. choleraesuis*	*S. enteritidis*	*P. aeruginosa*	*P. stuartii*	*A. calcoaceticus*	*B. subtilis*
Phormidium sp. (A)	Butanol	14.4±2.2	18.4±4	18.4±3	17.4±.3.5	24.3±3.5	22.6±1.2	24.6±2	9.4±0.8	18.6±2
	DCM	10.4±1	9.4±0.8	R	12.5±1.5	R	15.8±1.8	9.4±0.4	11.8±1.5	10.4±1
	Hexane	R	13.4±2	R	R	R	R	R	R	R
Chroococcus sp. (B)	Butanol	15.4±2.3	12.4±1.5	16±3	13.2±1	22.5±2.5	22.4±2.8	24.3±3	7.4±0	18.7±3.2
	DCM	8.4±0	10±1	R	10.4±0.95	11.4±1.2	R	11.4±0.5	13.3±1.2	11.4±0.8
	Hexane	R	R	R	R	R	R	R	R	R
Aphanizomenon sp.1	Butanol	16.4±.22	14.4±1.5	15.4±2.2	17.6±2.8	8.1±0	21.1±3	22.2±2	R	R
	DCM	R	10.4±0.8	R	9.3±0.8	9.4±0.5	R	10.5±0.75	13.6±1.5	NA
	Hexane	R	R	R	R	R	R	R	R	R
Aphanizomenon sp.2	Butanol	15.9±2	10.4±1	7.3±0	8.4±0.5	18.2±2	22.4±2.2	16±2	8.4±0.5	R
	DCM	9.4±1	8.4±0	9.2±0.8	10.2±1	10.4±1.2	11.5±0.95	3±0.25	11.6±0.8	11.4±0.5
	Hexane	R	R	R	R	R	R	R	R	R
Gleocapsa sp.(E)	Butanol	13.4±1.2	10.6±0.8	16.4±2	11.4±1.2	20.6±2	21.4±3.2	21.6±2.5	R	R
	DCM	10.4±0.5	9.4±0.3	11.4±0.56	9.3±0.2	12.2±1	15.5±1.1	12.6±1.5	R	8.4±0.5
	Hexane	R	9.4±0.3	R	R	R	R	R	R	R
	Butanol	13.4±0.5	11.4±1.2	19.3±0.56	12.8±0.2	22.5±1	19.4±1.1	20.5±1.5	R	8.5±0.5
Lyngbya sp. (F)	DCM	8.4±0.5	8.3±0.5	10.4±1.5	11.4±0.8	8.4±0.5	9.4±0.5	10.4±0.8	9.5±1	8.5±0.5
	Hexane	R	R	R	R	R	R	R	R	R
Leptolyngbya sp. (G)	Butanol	14.4±2.1	12.4±2.2	15.4±1.5	12.6±2.1	19.2±2.3	20.5±2.5	19.3±2	R	12.4±2.5
	DCM	8.4±0.5	9.3±0.5	10.6±1	8.2±0.5	10.4±1.5	11.4±1.5	11.4±2.1	8.4±0.5	9.5±1
	Hexane	R	R	R	R	R	R	R	R	R
Nostoc sp. 1 (H)	Butanol	11.4±0.95	18.4±2.1	14.4±1.8	21.5±1.6	14.5±2.2	20.3±3.2	19.4±2	R	8.4±0.5
	DCM	10.4±0.95	10.2±0.5	12.2±2.2	10.4±1.8	9.7±0.6	9.4±0.95	11.3±1.6	13.8±2.1	NA
	Hexane	R	R	R	R	R	R	R	R	R
Nostoc sp. 2 (I)	Butanol	13.5±1.6	16.4±1.5	16.4±.2.1	18.8±3.4	11.4±0.85	19.4±2	20.6±1.8	R	16.4±1.1
	DCM	12.4±2.2	13.4±1.5	12.8±2.5	11.2±1.6	11.4±1.5	13.8±2	8.4±0.5	10.4±0.95	NA
	Hexane	12.2±1.5	12.4±0.7	R	R	R	R	R	R	R

R: resistant and no inhibition zone is formed

NA: data not available

Table 4. Antifungal activity of cyanobacterial isolates. Data are the mean diameters of inhibition zones (mm). Cyanobacterial butanol, DCM and hexane extracts were applied to sterile paper disks (diameter = 6.4 mm).

	Fungal pathogens					
Cyanobacterial spp.	*Rhizoctonia solani*			*Phythium* sp.		
	Butanol	DCM	Hexane	Butanol	DCM	Hexane
Phormidium sp.	15.4	14.4	15.4	15.4	15.4	15.4
Gleocapsa sp.	15.4	15.4	15.4	15.4	15.4	15.4
Anabeana sp.1	15.4	15.4	15.4	15.4	15.4	15.4
Anabeana sp.	29.9	14.4	15.4	14.4	15.4	15.4
Chrocodiopsis sp.	15.4	15.4	15.4	15.4	15.4	15.4
Lyngbya sp.	15.4	15.4	15.4	15.4	15.4	15.4
Leptolyngbya sp.	14.7	15.4	15.4	15.3	15.4	15.4
Nostoc sp.1	10.4	15.4	15.4	15.4	15.4	15.4
Nostoc sp.2	15.4	15.4	15.4	15.4	15.4	15.4

The diameters of the inhibition zones were bigger than the antibacterial halos, revealing greater antifungal activity of the cyanobacterial extracts. Most of the extracts (in butanol, hexane and DCM) had similar activity (Table 3). Minimum activity was observed in strains collected from Bowsher hot spring. Various investigations have confirmed the antifungal properties of different cyanobacterial strains [20, 57-61]. Various antifungal compounds have been isolated from cyanobacteria including fisherellin A, hapalindole, carazostatin, phytoalexin, tolytoxin, scytophycin, toyocamycin, tjipanazole, nostocyclamide and nostodione [62], which supports the present findings.

4. Conclusion

The results obtained in the present investigation clearly demonstrate that cyanobacteria from thermal springs have strong antibacterial and antifungal properties. In particular, cyanobacterial isolates belonging to *Phormidium* and *Gleocapsa* genera have promising antibacterial and antifungal activities. Further knowledge of the composition, analysis and properties of these cyanobacteria with respect to antimicrobial compounds may be useful in various future biotechnological and pharmaceutical applications. Further research should be done, and bioactive compounds from these organisms should be isolated and investigated in the future.

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