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Nostoc piscinale Gt-319, a New Cyanobacterial Strain with Cytotoxic Activity

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Abstract: A total of 100 supernatants and methanol extract samples obtained from three weeks' incubation of 50 different isolate strains of cyanobacteria with paddy-fields origin were investigated for their cytotoxicity properties. Fifteen supernatants and 10 methanol extract samples exhibited cytotoxic activity against Balb-C cells while one methanol extract sample possessed a cytotoxic effect against five different cell lines including HeLa, Vero, Caco-2, HepG-2 and CHO more than vincristine, 5-fluorouracil and methotrexate when used at their IC_{50} concentrations. The later potent extract was achieved from the cyanobacterium strain GT-319. It was characterized as *Nostoc piscinale* Kützing ex Bornet and Flahault 1888 according to the morphological studies. Identification of the species was performed following molecular taxonomy by 16S rRNA sequencing. This is the first report on the cytotoxic activity of *N. piscinale* GT-319.

Key words: *Nostoc piscinale*, cyanobacteria, cytotoxicity, 16S rRNA, cell lines

INTRODUCTION

Investigation of the chemicals produced by macro- and microorganisms has resulted in the discovery of numerous bioactive natural compounds. Early studies, which focused on terrestrial plants and microorganisms, proved extremely fruitful, yielding many useful organic substances, for example, as many as 25% of the currently used anticancer substances, with another 25% coming from synthetic derivatives of natural products (Davidson, 1995; Kumar and Zi-Rong, 2004).

In the last decade, many reports have shown the potential of cyanobacteria in producing a variety of bioactive compounds (Burja *et al*., 2001; Mundt *et al*., 2001). Toxic cyanobacteria have been also recognized as a worldwide hygienic problem in recent years (Lee *et al*., 1999). Despite a growing awareness of the presence of cyanobacterial toxins, knowledge regarding the ability of specific species to produce toxic compounds is still rather limited (Teneva *et al*., 2003).

Some of the cyanobacterial metabolites, in particular, extra-cellular ones, possibly function as toxins (Parti *et al*., 2002). In addition, many active substances that perform antibacterial, antiviral, fungicide, enzyme-inhibiting, immunosuppressive, cytotoxic and algaecide activities have been isolated from cyanobacterial biomasses or supernatants (Kaebernick and Neilan, 2001). However, most of the research in this area has been focused on the pharmaceutical applications of cyanobacteria-derived products (Lieberman *et al*., 2001; Kreitlow *et al*., 1999).

Until now, a variety of cytotoxic compounds such as cryptophycins (Shih and Teicher, 2001), tasiamide (Williams *et al*., 2002), tasipeptins A and B (Williams *et al*., 2003a), ulongapeptin (Williams *et al*., 2003b), micromidem and guamamide (Williams *et al*., 2004), microcyclamide (Ishida *et al*., 2000), cylindrospermopsin (Runnegar *et al*., 1995) and nostocine A (Hirata *et al*., 2003, 2004), respectively, have been isolated from various strains of cyanobacteria. These kinds of microorganisms can be also considered as a source of new lead compounds, because they probably produce a wide range of novel and biologically active natural products (Runnegar *et al*., 1995).

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Secondary metabolite content and therefore, biological activity of cyanobacteria depends on environmental conditions (Ghasemi *et al*., 2003, 2004). Paddy-fields cyanobacteria contain valuable genera to produce bioactive metabolites (Golakoti *et al*., 2000; Hirata *et al*., 2003). In a previous study, we reported the importance of Iranian paddy-fields cyanobacteria, which have potent antibacterial and antifungal activities (Ghasemi *et al*., 2003, 2004). The ability of the same cyanobacteria in steroid substance biotransformation was also recently shown (Faramarzi *et al*., 2006; Moradpour *et al*., 2006; Tabatabaei Yazdi *et al*., 2004).

However, the cytotoxic activity of Iranian paddy-fields cyanobacteria has not been studied up to now. In the present study, 50 different paddy-fields cyanobacteria were isolated and investigated for their ability to produce cytotoxic compounds. The aim of this study is to isolate and identify the most potent strain and compare its cytotoxic activity with known pharmaceutically cytotoxic substances.

MATERIALS AND METHODS

Materials: All chemicals and solvents were purchased from Merck. The following cell lines were purchased from the Pasteur Institute of Iran. Vero (NCBI-C101), Caco-2 (NCBI-C139), CHO (NCBI-C111), Hela (NCBI-C115), HepG2 (NCBI-C158) and Balb-C cells were maintained in the Department of Toxicology, Faculty of Pharmacy, Tehran University of Medical Sciences and freshly subcultured before use in the cytotoxicity studies. Methotrexate, 50 mg mL^{-1} (Ebewe), vincristine, 1 mg/10 mL (Gedeon Richter Ltd.) and 5-fluorouracil (5-FU), 250 mg/5 mL (Roche) were the cytotoxic reference substances.

Isolation of cyanobacteria: Soil samples were collected from different paddy-fields of Iran (mainly from the north and southwest parts) in June 2005. The samples were inoculated directly in BG-11 liquid media. After colonization, cyanobacteria were transferred to the fresh media. Fifty unialgal strains were isolated and then purified using agar plate method (Ghasemi *et al*., 2003). Purified cyanobacteria were maintained at 4°C on a BG-11 (Borowitzka, 1988) agar slant and freshly subcultured before use in the cytotoxicity experiments. The organisms were transferred to fresh media every two months.

Preparation of extracts: The cyanobacteria were individually inoculated in 50 500 mL-Erlenmeyer flasks containing 100 mL of BG-11 liquid media, illuminated continuously with fluorescent lamps at 40 $\mu Em^{-2} S^{-1}$ and incubated at a temperature of 25±2°C without shaking for three weeks.

After three weeks of incubation, the cells were harvested by filtration and the supernatant was concentrated on a rotary evaporator apparatus under reduced pressure to reach the volume of 5 mL. The supernatant was then considered for the cytotoxicity evaluation experiments. The biomass was washed with water and extracted using 30 mL of methanol while being shaken for 20 min. The methanol extract was then filtered and concentrated to 1 mL under reduced pressure. The concentrated extracts and supernatants were sterilized by passing them through a 0.22 μm filter before use in the cytotoxicity evaluation.

Cell culture and treatment: The Balb-C cells were grown in Dulbecco, Modified Eagle's Medium (DMEM) supplemented with 2 mM L-glutamine, 10% fetal bovine serum and 1% antibiotic solution containing 10000 U L^{-1} penicillin and 5 mg L^{-1} streptomycin. The cells were incubated at 37°C and 5% CO_2 in a humidified incubator.

CHO, Vero, Hela, Caco-2 and HEPG-2 cells were cultivated in RPMI medium supplemented with 10% FBS and 1% antibiotic solution. After two days, the cell cultures were trypsinized to obtain single cell suspensions. The viability of the cells was determined by trypan blue dye exclusion. Twenty microliter of 0.5% v/v trypan blue was added to the 20 μL cell suspension and the cells not stained by the dye were counted (Ostad *et al*., 1998). Fifty thousand cells were cultured in each well of the 24 well dishes and incubated at the same condition. After 24 h, the cells were treated with 50 μL of each extracts and at the same time, Phosphate Buffer Saline (PBS) and reference cytotoxic substances were used as the negative and positive controls, respectively. All experiments were performed at least three times and three wells used for each extract.

Cytotoxicity assay: Cytotoxicity test with Neutral red stain was applied to measure cell viability. The test is based on the lysosomal adding of supravital neutral red stain which quantifies the number of viable cells after exposure to a physical or chemical agent. The quantification of the stain extracted from cultured cells has been shown to be linear with the number of viable cells through direct count (Babich *et al*., 1991). Neutral red (200 μL of 4% solution) was added to each well and incubated for 90 min at 37°C. After the incubation period, the dye was removed and the cells were washed three times with pre-warmed PBS. Dissolving solution (absolute ethanol/glacial acetic acid/ water; 50:1:49; v/v/v) was added to each well and the plates were agitated for 20 min at room temperature. The optical density was measured at 540 nm using a plate reader (Stat Fax®, USA) (Ostad *et al*., 2004). The Cytotoxicity percentage is calculated as [(A-B)/ (A-C)] ×100, where A and B are the OD_{540} of untreated and treated cells and C is OD_{540} treated cells with reference cytotoxic substances, respectively (Betancur *et al*., 2002).

The differences between the percents of cytotoxicity were evaluated by analysis of variance (ANOVA).

Identification of cyanobacteria: Identification of the isolate cyanobacteria was done using morphological studies and taxonomical approaches with preparation semi-permanent slides from each specimen according to Prescott (1962), John (2002) and Anagnostidis and Komarek (1988) identification keys. The slides were coded and preserved in the Algal Herbarium of Applied Science Centre, Jahad Daneshgahi, Shahid Beheshti University, Tehran, Iran.

16S ribosomal RNA sequencing: The sequence of 16S ribosomal RNA of the selected strain was studied. For this purpose, DNA content was first extracted from the cyanobacterium and then PCR was applied using two sets of primers. The primers specified for 16S rRNA of cyanobacterium used 5'GGGGAATCTTCCGCAATGGG3' as forward (CYA 359 F) and 5'GACTACAGGGGTATCTAATCC3' as reverse (CYA 781 R) primers (Nubel *et al*., 1997), respectively.

To extract DNA from the cyanobacterium, a fresh biomass was obtained by centrifugation at 14000 rpm. After washing the biomass two times with PCR distillated water, it was incubated at 96°C for 10 min and then centrifuged at 14000 rpm. The supernatant was used as a template for PCR. The applied PCR condition has been described by Nubel *et al*. (1997). Agarose 1% (Invitrogen) in TAE buffer was used to evaluate the PCR product. A single 442 bp band of DNA (Fig. 1) was cut and extracted from the gel using the QIAquick Gel Extraction kit. The sequence was determined by the FazaBiotech Company with CYA 359 F and CYA 781 R primer.

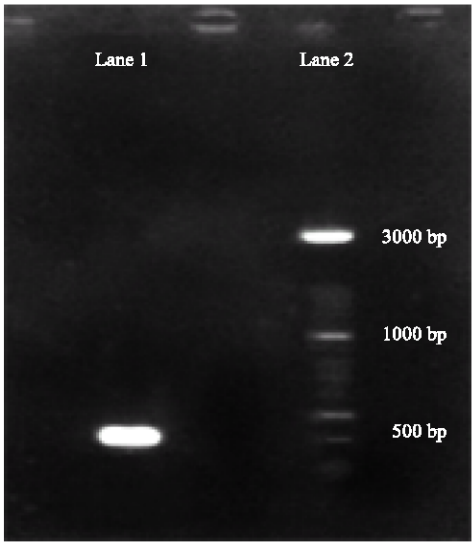

Fig. 1: PCR product (Lane 1) representing with size markers (Lane 2)

BLAST search: Sequence similarity searches were done with BLAST through the website of the NCBI.

RESULTS

Primary screening: As was shown in Table 1, 15 supernatants and 10 methanol extract samples obtained from cultivated cyanobacteria showed cytotoxic activity more than methotrexate.

These organisms belonged to five cyanobacterial families: Chroococcaceae (strains GT-1, GT-14 and GT-15), Microchaetaceae (strain GT-2), Stigonemataceae (strains GT-3, GT-4, GT-6, GT-7, GT-9, GT-11, GT-12, GT-13, GT-17, GT-526, GT-385, P-351, P-402, P-383, P-322, GT-185, P-357, GT-271, GT-367, GT-330, GT-370, GT-356 and GT-531), Nostocaceae (strains GT-5, GT-8, GT-16, GT-317, P-369, GT-355, GT-366, GT-361, GT-352, P-4, P-3, GT-319, GT-359, P-358, P-353, GT-364, GT-365 and GT-354) and Oscillatoriaceae (strains GT-10, P-368, GT-362, GT-360 and P-374), respectively.

Out of 100 crude extracts corresponding to 50 different cyanobacterial strains, 15 supernatants showed cytotoxicity to equal or more than methotrexate when used at its IC_{50}, 33 supernatants had poor cytotoxicity effects and 2 supernatants did not show any cytotoxicity property. In the methanol extract samples, 10 extracts showed considerable cytotoxicity more than methotrexate at its IC_{50} concentration, other methanol extracts had weak cytotoxicity and 1 extract had no cytotoxicity.

Supernatants and methanol extracts that were obtained from the strains of *Nostoc* species showed potent cytotoxic activities and of the methanol extract and the supernatant, the supernatant of these strains had better cytotoxic activity.

Secondary screening: In the second screening step, 6 strains, in which both supernatants and methanol extract samples showed cytotoxicity effects more than methotrexate, were selected for further evaluation. Therefore, their cytotoxicity was evaluated against other cell lines and compared with other cytotoxic substances. As shown in Table 2-4, both supernatant and methanol extracts that belonged to the strain of *Nostoc piscinale* GT-319 showed higher cytotoxicity against all the cell lines in comparison with vincristine, methotrexate and 5-FU.

Table 1: Cytotoxicity percent of extracts against Balb-C cells in comparison with methoterxate as positive reference

No.	Strain	Cyanobactrium family	Supernatant cytotoxicity (%)	Methanol extract cytotoxicity (%)
1	*Chroococcus* sp. GT-1	Chroococcaceae	45.0±4	074±4
2	*Toilypothrix* sp. GT-2	Microchaetaceae	8.1±2	112±10*
3	*Hapalosiphon* sp. GT-3	Stigonemataceae	31.0±2	024±2
4	*Hapalosiphon* sp. GT-4	Stigonemataceae	31.0±3	075±4
5	*Nostoc* sp. GT-5	Nostocaceae	16.0±2	026±5
6	*Hapalosiphon* sp. GT-6	Stigonemataceae	126.0±9*	037±2
7	*Hapalosiphon* sp. GT-7	Stigonemataceae	72.0±4	004±1
8	*Nostoc* sp. GT-8	Nostocaceae	89.0±5	031±3
9	*Stigonema* sp. GT-9	Stigonemataceae	26.0±2	090±5
10	*Plectonema* sp. GT-10	Oscillatoriaceae	70.0±6	032±5
11	*Stigonema* sp. GT-11	Stigonemataceae	0.0	022±2
12	*Stigonema* sp. GT-12	Stigonemataceae	0.0	015±3
13	*Fischerella* sp. GT-13	Stigonemataceae	34.0±3	003±1
14	*Chroococcus* sp. GT-14	Chroococcaceae	59.0±4	045±4
15	*Chroococcus* sp. GT-15	Chroococcaceae	120.0±6*	062±3
16	*Nostoc* sp. GT-16	Nostocaceae	142.0±5*	038±3
17	*Hapalosiphon* sp. GT-17	Stigonemataceae	86.0±2	058±2
18	*Fischerella* sp. GT-526	Stigonemataceae	130.0±9*	008±1
19	*Stigonema* sp. GT-385	Stigonemataceae	100.0±10*	042±2
20	*Nostoc* sp. GT-317	Nostocaceae	35.0±3	004±1
21	*Nostoc* sp. P-369	Nostocaceae	69.0±2	105±5*
22	*Stigonema* sp. P-351	Stigonemataceae	35.0±3	049±6
23	*Stigonema* sp. P-402	Stigonemataceae	64.0±4	086±6
24	*Hapalosiphon* sp. P-383	Stigonemataceae	54.0±3	120±9*
25	*Fischerella* sp. P-322	Stigonemataceae	116.0±6*	077±3
26	*Nostoc* sp. GT-355	Nostocaceae	117.0±10*	064±4
27	*Nostoc* sp. GT-366	Nostocaceae	113.0±3*	111±10*
28	*Nostoc* sp. GT-361	Nostocaceae	139.0±10*	128±10*
29	*Nostoc* sp. GT-352	Nostocaceae	132.0±10*	133±10*
30	*Nostoc* sp. P-4	Nostocaceae	128.0±5*	158±10*
31	*Nostoc* sp. P-3	Nostocaceae	129.0±10*	107±5*
32	*Nostoc* sp. GT-319	Nostocaceae	130.0±10*	117±10*
33	*Fischerella* sp. GT-185	Stigonemataceae	28.0±3	023±2
34	*Nostoc* sp. GT-359	Nostocaceae	55.0±5	068±6
35	*Oscillatoria* sp. P-368	Oscillatoriaceae	59.0±5	026±4
36	*Nostoc* sp. P-358	Nostocaceae	57.0±4	031±3
37	*Stigonema* sp. P-357	Stigonemataceae	38.0±6	017±3
38	*Nostoc* sp. P-353	Nostocaceae	60.0±5	068±3
39	*Fischerella* sp. GT-271	Stigonemataceae	50.0±5	059±5
40	*Oscillatoria* sp. GT-362	Oscillatoriaceae	57.0±2	111±9*
41	*Stigonema* sp. GT-367	Stigonemataceae	30.0±3	058±5
42	*Nostoc* sp. GT-364	Nostocaceae	67.0±4	060±4
43	*Hapalosiphon* sp. GT-330	Stigonemataceae	53.0±3	012±1
44	*Nostoc* sp. GT-365	Nostocaceae	153.0±10*	048±4
45	*Oscillatoria* sp. GT-360	Oscillatoriaceae	98.0±2	000
46	*Nostoc* sp. GT-354	Nostocaceae	69.0±5	017±2
47	*Fischerella* sp. GT-370	Stigonemataceae	190.0±10*	052±2
48	*Stigonema* sp. GT-356	Stigonemataceae	65.0±5	031±2
49	*Oscillatoria* sp. P-374	Oscillatoriaceae	23.0±2	079±5
50	*Hapalosiphon* sp. GT-531	Stigonemataceae	74.0±4	096±3

*The percent of cytotoxicity indicates significant difference between the data ($p<0.05$) by ANOVA

Table 2: Cytotoxicity percent of the extracts against different cell lines in comparison with vincristine (45 nM)* as positive reference

Strain	Extract	HepG-2	Hela	CHO	Vero	Caco-2
GT-366	Supernatant	0.0	28.0±2	79.0±5	18.0±3	35±5
GT-366	Methanol extract	50.0±5	0.0	0.0	0.0	69±4
GT-361	Supernatant	46.0±4	0.0	0.0	52.0±5	54±3
GT-361	Methanol extract	34.0±4	29.0±5	36.0±4	0.0	95±4
P-3	Supernatant	76.0±3	43.0±2	92.0±5	54.0±4	11±2
P-3	Methanol extract	79.0±5	0.0	0.0	0.0	82±4
P-4	Supernatant	0.0	43.0±3	23.0±2	34.0±2	42±3
P-4	Methanol extract	41.0±2	41.0±2	0.0	26.0±3	26±5
GT-319	Supernatant	72.0±4	83.0±5	110.0±5	50.0±4	110±5
GT-319	Methanol extract	105.0±5	116.0±5[a]	116.0±4[a]	112.0±4	99±5
GT-352	Supernatant	51.0±2	48.0±5	88.0±4	113.0±3	111±5
GT-352	Methanol extract	42.0±3	70.0±5	107.0±5	33.0±3	88±5

[a]Indicates significant difference between the data ($p<0.05$) by ANOVA; *IC_{50} of Vincristine according to Terashi *et al.* (2000)

Table 3: Cytotoxicity percent of the extracts against different cell lines in comparison with methotrexate (20 nM)* as positive reference

Strain	Extract	HepG-2	Hela	CHO	Vero	Caco-2
GT-366	Supernatant	124±10	026±5	100±5	039±3	0
GT-366	Methanol extract	101±6	0	0	0	052±3
GT-361	Supernatant	146±8[a]	074±4	0	0	048±5
GT-361	Methanol extract	138±10	049±5	064±3	039±5	035±3
P-3	Supernatant	094±4	082±3	130±10	058±4	080±3
P-3	Methanol extract	120±10	0	0	0	082±5
P-4	Supernatant	132±10	048±5	033±5	059±6	0
P-4	Methanol extract	038±5	037±4	0	056±4	043±3
GT-319	Supernatant	133±5	056±4	155±5[a]	112±4	076±3
GT-319	Methanol extract	144±5[a]	113±5	164±8[a]	157±5[a]	110±5
GT-352	Supernatant	135±4	113±5	124±6	064±3	053±5
GT-352	Methanol extract	128±10	033±4	151±10[a]	095±5	044±2

[a]Indicates significant difference between the data ($p<0.05$) by ANOVA; *IC_{50} Methoterexate according to Wosikowski *et al.* (2003)

Table 4: Cytotoxicity percent of the extracts against different cell lines in comparison with 5-FU (12 μM)* as positive reference

Strain	Extract	HepG-2	Hela	CHO	Vero	Caco-2
GT-366	Supernatant	092±4	026±3	078±3	036±2	0
GT-366	Methanol extract	070±5	0	0	0	53±4
GT-361	Supernatant	049±5	076±4	0	0	49±5
GT-361	Methanol extract	097±5	050±3	033±1	036±2	35±3
P-3	Supernatant	070±4	083±5	086±3	054±5	83±3
P-3	Methanol extract	084±3	0	0	0	85±5
P-4	Supernatant	098±5	049±4	022±3	055±5	0
P-4	Methanol extract	026±4	038±3	0	051±5	44±3
GT-319	Supernatant	100±5	073±2	102±2	104±2	79±4
GT-319	Methanol extract	101±1	164±5[a]	108±4	145±5[a]	115±5
GT-352	Supernatant	101±5	164±10[a]	082±5	059±4	55±5
GT-352	Methanol extract	090±5	048±4	100±5	088±6	45±5

[a]Indicates significant difference between the data ($p<0.05$) by ANOVA; *IC_{50} 5-FU according to Tao *et al.* (2004)

Identification: In morphological studies, the cyanobacterium strain GT-319 showed green to golden brown thallus, sometimes with free visible filaments around the mother colonies: Globose akinetes, with an outer layer and granulate [8-9×8-8.2]; spherical to ovate vegetative cell [4-4.9×4]; rare and oblong heterocyst. Therefore, the cyanobacterium strain was considered to be a strain of *Nostoc piscinale* Kützing ex Bornet and Flahault 1888 belonging to the family of Nostocaceae.

The partial sequence of the 16S rRNA sequence of this species is as follows: 5'AGCATGATGACATACCGCGTGAGTGAGGAGGCTC TTGGAGTTGTAAACCTCTTTTCTCAGGGAATAAGA ACTGAAGGTACCTGAGGAATAAGCATCGGCTAAC TCCGTGCCAGCAGCCGCGGTAATACGGAGGATGCA AGCGTTATCCGGAATGATTGGGCGTAAAGGGTCCG CAGGTGGCCTTGTAAGTCTGCTGTTAAAGAGTGAG GCTTAACCTCATAAAAGCAGTGGAAACTACAAA GCTAGAGTGCGTTCGGGGTAGCAGGAATTCCTGGTG TAGCGGTGAAATGCGTATAGATCAGGAAGAACAC CGGTGGCGAAAGCGTGCTACTAGGCCGTAACTGAC ACTGAGGGACGAAAGCTAGGGGAGCGAATGGGAT TAGATACCCCCTGTAGACATC3'.

The 16S rRNA gene sequence alignment contained 106 characters, which did not have 100% homology with any submitted sequences.

The closest homology was found in comparison with *Nostoc* sp. KU001 with 97% identity and *Nostoc* sp. YK-01 with 95% identity, respectively. Other characters showed 94 or 93% identity and most belonged to the family of Nostocaceae.

Sequence accession number: The DNA sequence of *Nostoc piscinale* strain GT-319 was deposited in the GenBank under the accession number DQ837591 (16S rRNA).

DISCUSSION

Screening programs performed on crude extracts obtained from various types of microorganisms have been proved to be effective methods for introducing organisms that produce potentially useful compounds (Harrigan and Goetz, 2002; Skulberg, 2000). In this area, cyanobacteria were identified as the most promising groups of organisms from which to isolate novel, biochemically active, natural products.

In the study by Jaki *et al.* (1999) total of 86 extracts obtained from 43 samples of cyanobacteria were screened for their biological activities and it was found that almost 8.1% showed cytotoxic activities against Caco-2 cells and 1.2% against KB cells. Mian *et al.* (2003) evaluated a total of 44 extracts of cyanobacteria for their biological activities and

found that 38.6% of the extracts had cytotoxic effects against KB cells. Of 22 cyanobacteria investigated in this study, 19 were active in at least one of the applied antibacterial or cytotoxic assays, respectively. The study conducted by Pietsh *et al.* (2001) found that a cyanobacterial crude extract caused stronger cytotoxic effects than the pure cyanobacterial toxins used in equivalent concentrations. Kreitlow *et al.* (1999) conducted a study in which, among 12 cyanobacterial strains, 6 strains produced substances with antibacterial or cytotoxic activities. Because the study was done at an early stage, differentiation between cytotoxic and antibiotic substances was not possible. There have also been some reports on the biological activity of Iranian paddy-fields microalgae (Ghasemi *et al.*, 2003, 2004); however, the cytotoxic effects of existing cyanobacteria have not been studied up to now.

In the present study, a total of 100 different extracts from 50 cyanobacteria, which were isolated from soil habitats in different parts of Iran, were evaluated for their cytotoxic activities. Twenty-five of either the supernatants or methanol extracts showed cytotoxicity properties in comparison with methotrexate against Balb-C fibroblast cells. Among the applied extracts, 6 stains, in which their supernatant and methanol extracts showed cytotoxic activities, were selected for further evaluation on 5 more cell lines. Among these cell lines, CHO cells were the most sensitive to the extracts. Both methanol and supernatant extracts of strain GT-319 exhibit more cytotoxic activities to these cells in comparison with vincristine, methotrexate and 5-FU. All the cyanobacteria were toxic to the HepG-2 cells, when their cytotoxic activities were compared to methotrexate. None of the extracts were active against Vero cells except the extracts obtained from strain GT-319, when its activity was compared to vincristine and methotrexate. Extracts from strain GT-319 were the most active among other cyanobacteria against all of the cell lines when compared to standard cytotoxic substances.

Identification of the strain revealed that it belongs to the species of *Nostoc piscinale*. The result obtained from the 16S rRNA sequence BLAST of *Nostoc piscinale* GT-319 showed that there was not a 100% identity with the obtained sequence in the NCBI-submitted sequences, so the cyanobacterium could be a new strain of *Nostoc piscinale*. Its sequence was submitted to the GenBank under the accession number DQ837591. Nostoc strains have been known for their potency in producing valuable metabolites with antibacterial, antifungal, antiviral and pesticide activity (Biondi *et al.*, 2004; Piccardi *et al.*, 2000; Teneva *et al.*, 2003). Some cytotoxic metabolites have also been isolated from different species of *Nostoc*, such as cryptophycins (Ghosh and Bischoff, 2004; Shih and Teiche, 2001; Smith *et al.*, 2003), nostocine A (Hirata *et al.*, 2003, 2004) and nostopeptolides A_1, A_2, A_3 (Golakoti *et al.*, 2000).

In this study, *Nostoc piscinale* GT-319 was found to be a potent source for production of cytotoxic compound(s) and could be used as a new source for further purification and identification studies of active substance(s).

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