Tropical Journal of Pharmaceutical Research November 2018; 17 (11): 2183-2188
ISSN: 1596-5996 (print); 1596-9827 (electronic)

Available online at http://www.tjpr.org
http://dx.doi.org/10.4314/tjpr.v17i11.11

Original Research Article

Anticancer potential and cytotoxic effect of some freshwater cyanobacteria

Tunay Karan*, Ali Aydin
Department of Molecular Biology and Genetics, Faculty of Arts and Sciences, Gaziosmanpasa University, 60240 Tokat, Turkey

For correspondence: **Email: biyo_tunay@hotmail.com; **Tel:** +90 356 252 16 16*

Sent for review: 31 March 2018 *Revised accepted: 25 October 2018*

Abstract

Purpose: *To investigate the anticancer potential and cytotoxicity of some freshwater cyanobacterial extracts on Human Colon Carcinoma (HT29), Rat Brain Glioma (C6), Human Cervix Carcinoma (HeLa), Human Lung Carcinoma (A549), Human Breast Adenocarcinoma (MCF7), Human Liver Hepatocellular Carcinoma (Hep3B) cancerous cells, African Green Monkey Kidney cells (Vero), and Human Amnion cells (FL).*
Methods: *Chroococus minutus, Geitlerinema carotinosum, Nostoc linckia and Anabaena oryzae were collected from different freshwater habitats and identified. Each cyanobacterium was extracted with methanol. Antiproliferative activities of the cyanobacterial extracts were evaluated by [3-(4, 5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide] (MTT) using HT29, C6, HeLa, A549, MCF7, Hep3B, Vero, and FL cell lines. Cytotoxicity was determined by lactate dehydrogenase (LDH) assay.*
Results: *The cyanobacterial extracts showed varying antiproliferative and cytotoxic effects on cancer cells. G. carotinosum and N. linckia had significant inhibitory effect on C6 cell lines with half-maximal inhibitory (IC_{50}) levels of 112.69 and 121.48 µg/mL, respectively. TGI values for G. carotinosum and N. linckia were 65.07 and 70.61 µg/mL, respectively. LC_{50} values for these two cyanobacteria were 386.64 and 760.55 µg/mL, respectively. In addition, cyanobacterium A. oryzae displayed excellent antiproliferative effect on MCF7 cancer cells (GI_{50} = 2.04 µg/mL). The extracts displayed the cytostatic effect on the cell lines.*
Conclusion: *G. carotinosum, N. linckia and A. oryzae exhibit significant activity on various cancer cells. Hence these cyanobacteria may offer promise as anticancer agents.*

Keywords: *Freshwater cyanobacteria, Anticancer, Cytotoxicity, Chroococus minutus, Geitlerinema carotinosum, Nostoc linckia, Anabaena oryzae*

Tropical Journal of Pharmaceutical Research is indexed by Science Citation Index (SciSearch), Scopus, International Pharmaceutical Abstract, Chemical Abstracts, Embase, Index Copernicus, EBSCO, African Index Medicus, JournalSeek, Journal Citation Reports/Science Edition, Directory of Open Access Journals (DOAJ), African Journal Online, Bioline International, Open-J-Gate and Pharmacy Abstracts

INTRODUCTION

Cyanobacteria, known as cyanoprokaryotes or blue-green bacteria, and gram negative prokaryotes are some of the oldest living forms in the world, dating back to 3.7 billion years [1, 2]. The cyanobacteria have significant activities arising from production of some bioactive secondary metabolites [3]. Filamentous sea cyanobacteria have been reported to prevent cancer growth, neurodegenerative and infectious diseases [4]. Sea cyanobacteria include a great variety of compounds with pro-apoptotic effects [5]. Sea cyanobacteria *Synechocystis* sp. and

Synechococcus sp. were reported to inhibit human promyeloctic leukemia (HL-60) cells [6]. Rise of cancer incidence and limitations of existing drugs make it desirable to develop new anticancer molecules from various sources including freshwater cyanobacteria [7]. It was reported that water extracts of *Nostoc muscorum* and *Oscillatoria* sp. showed high anticancer activity against Ehrlich Ascites Carcinoma cell and Human Hepatocellular (HepG2) cells [8]. In addition, aqueous extract of *Spirulina* sp. have been shown to inhibit Hepatic Stellate cell (HSC). Besides, crude extracts of six cyanobacteria (*Phormidium* sp., *Geitlerinema* sp., *Arthrospira* sp., *Phormidium* sp. *Phromidium* sp. and *Leptolyngbya* sp. demonstrated concentration-dependent inhibitions of Homo Sapiens Kidney Carcinoma and Homo Sapiens Colon Colorectral Adenocarcinoma [9]. Similarly, cyanobacterium *Cyanothece* sp. had high anticancer effect on extracellular T-lymphoma cells [10].

In the present study, anticancer properties of freshwater cyanobacteria species (*Chroococcus minutus*, *Geitlerinema carotinosum*, *Nostoc linckia*, Anabaena *oryzae*) were investigated on HeLa, HT29, A549, MCF7, Hep3B, C6 cancer cells and FL, Vero cells.

EXPERIMENTAL

Isolation and culture of cyanobacteria

Cyanobacteria were collected from freshwater habitats around Tokat Yesilirmak River. Collected and identified cyanobacteria were isolated mechanically under an inverted microscope. The cyanobacteria *C. minutus* and *G. carotinosum* were grown in a 250 ml Erlenmeyer consisting of Bristol medium (125 mL). *N. linckia* and *A. oryzae* were cultured in an Erlenmeyer vessel containing 125 ml BG-11 medium [11]. The cells in the vessel were incubated under fluorescent lamp at 26 ± 2 °C for 15 days. Then, cyanobacteria were centrifuged and dried at 60 °C.

Cyanobacteria extraction

Each dry cyanobacterium was extracted with methanol (50 mL) for 48 h. The solvent was filtrated and then removed by rotary evaporator to yield the extract which was subsequently dissolved in dimethyl sulfoxide (DMSO) (1.5 mL) for storage at 4 °C until use [12].

Cell lines

HeLa, MCF-7, Hep3B, A549, C6, HT29 cancer cell lines, along with FL cell and Vero kidney epithelial cell were used. Cell lines were incubated on a medium (Dulbecco's Modified Eagle Medium) enriched with 10% fetal bovine serum and 2% penicillin Streptomycin at 37 °C and 5% CO_2 environment. Analyses were carried out after cells reached a certain density.

MTT cell proliferation assay

MTT test was used to evaluate the effects of extracts on cell proliferation [13]. After exposing cancer cells to the extracts for 24 h, MTT test was performed.

A stock solution was prepared by mixing 5 mg/ml MTT solution with RPMI1640 medium (without phenol red). This new solution was added to the reaction medium with incubation for 4 h. Absorbance measurement was carried out by a spectrophotometer at 560 nm.

Calculation of GI_{50}, TGI, LC_{50} and IC_{50} parameters

At the end of incubation, cell inhibition was calculated. The half-maximal inhibitory concentration of the extracts (IC_{50}) and 5FU were calculated by XLfit5 software as in Eq 1 [14].

$$\text{Inhibition (\%)} = \{(A - B)/B\} \times 100 \quad \ldots\ldots\ldots (1)$$

where A is the absorbance of the extracts and B absorbance of untreated cells.

GI_{50} (50 of % Growth inhibition), TGI_{50} (Total growth inhibition) and LC_{50} (50% of drug causing cell kill) parameters were calculated for the extracts using Eqs 2 and 3.

$$GI_{50} = \{(Ti-Tz)/(C-Tz)\} \times 100 = 50 \text{ (provided that } Ti \geq Tz) \ldots\ldots\ldots (2)$$

$$TGI\ Ti = Tz \ldots\ldots\ldots (3)$$

$$50\% \text{ of drug causing cell kill } (LC_{50}) = \{(Ti-Tz)/Tz\} \times 100 = -50 \text{ (provided that } Ti < Tz) \ldots (4)$$

Here; Tz; zero point, C; control growth, Ti; inhibition of the test substance, TGI: Total growth inhibition [15].

Determination of cytotoxic effects

Lactate dehydrogenase (LDH) assay was carried out based on procedure provided by the manufacturer (Roche, USA). LDH activity was measured in terms of the amount of formazan produced. LDH activity was determined as absorbance at 492 - 630 nm by a microplate

reader (RAYTO RT100C). Cytotoxic activity (T) was determined using Eq. 5 [14,15].

T (%) = {(EV - LC/HC - LC)}100 …..... (5)

where EV = experimental value, LC = low control and HC = high control.

Statistical analysis

The experiments were performed in triplicate and all data are expressed as mean ± standard deviation (SD). The data were analyzed by one-way ANOVA using SPSS software (version 21.0).

RESULTS

Anticancer activity

GI_{50}, TGI, LC_{50} and IC_{50} values of *C. minutus* and *G. carotinosum* were given in Table 1. The antiproliferative activity of *N. linckia* and *A. oryzae are* shown in Table 2. Anticancer drug 5FU was used as a positive control (Table 3).

Compared with IC_{50} values of 5FU and cyanobacterial extracts, *G. carotinosum* and *N. linckia* showed better activity against C6 cell lines (112.69 ± 0.07 µg/mL and 121.48 ± 0.09 µg/mL, respectively) than 5FU did (134.67 ± 1.02 µg/mL).

Table 3: GI_{50}, TGI, LC_{50}, and IC_{50} values for 5FU

Cell line	5FU			
	GI_{50}	***TGI***	***LC_{50}***	***IC_{50}***
A549	13.34	63.25	763.06	83.62
HeLa	15.68	60.91	461.93	55.13
Hep3B	15.31	91.28	>1000	161.13
HT29	22.40	94.23	769.66	78.93
MCF7	7.28	47.47	>1000	48.42
C6	21.82	102.72	>1000	134.67
Vero	20.01	93.73	972.29	86.45
FL	21.23	82.36	582.62	138.38

In terms of 50 % growth inhibition parameter (GI_{50}), *C. minutus* significantly inhibited growth of Hep3B and C6 cancer cells at 18-20 µg/mL. It also inhibited the growth of MCF7 cells (3.31 ± 1.02 µg/mL) better than standard 5FU did (7.28 ± 0.92 µg/mL). *G. carotinosum* successfully inhibited growth of the MCF7 and C6 cells as well. *N. linckia* and *A. oryzae* extracts had a significant effect to inhibit MCF7, Hep3B and C6 cancer cell lines. Indeed, *A. oryzae* extract displayed the highest growth inhibition on MCF7 cells with a value of 2.04 ± 0.32 µg/mL.

G. carotinosum and *N. linckia* extracts inhibited total growth of the C6 cell at 65.07 ± 0.23, 70.61 ± 0.45 µg/mL, respectively, which were better than 5FU. *A. oryzae* also had an inhibition effect on C6 cell (103.01 ± 0.12 µg/mL) close to 5 FU. A comparison with the standard (5FU) showed that the extracts had considerable levels of antiproliferative effects with potential to be used in pharmacology.

Table 1: GI_{50}, TGI, LC_{50} and IC_{50} for *C. minutus* and *G. carotinosum*

Cell line	*C. minutus*				*G. carotinosum*			
	GI_{50}	**TGI**	**LC_{50}**	***IC_{50}**	**GI_{50}**	**TGI**	**LC_{50}**	***IC_{50}**
A549	83.11	>1000	>1000	389.93	44.75	>1000	>1000	284.20
HeLa	35.95	752.93	>1000	209.43	27.60	267.95	>1000	170.74
Hep3B	19.81	>1000	>1000	296.15	21.77	801.54	>1000	296.07
HT29	240.77	>1000	>1000	367.78	89.93	>1000	>1000	330.17
MCF7	3.31	253.69	>1000	163.46	3.43	211.87	>1000	148.58
C6	18.61	136.08	>1000	164.90	18.66	65.07	386.64	112.69
Vero	112.52	>1000	>1000	353.95	45.18	227.56	>1000	144.97
FL	17.84	458.51	>1000	274.44	20.10	189.01	>1000	227.37

*Linear equation was used

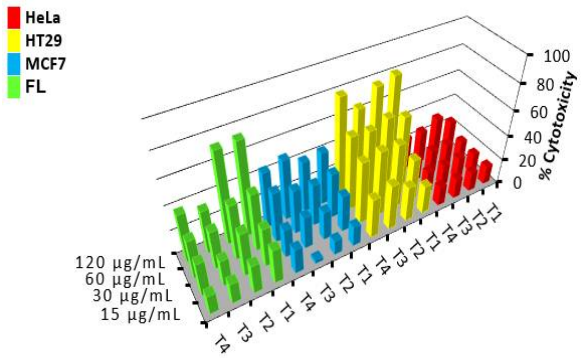
Table 2: GI_{50}, TGI, LC_{50} and IC_{50} for *N. linckia* and *A. oryzae*

Cell line	*N. linckia*				*A. oryzae*			
	GI_{50}	***TGI***	***LC_{50}***	***IC_{50}**	***GI_{50}***	***TGI***	***LC_{50}***	***IC_{50}**
A549	143.60	>1000	>1000	370.84	248.92	>1000	>1000	407.95
HeLa	18.38	214.06	>1000	151.36	19.81	253.49	>1000	168.38
Hep3B	14.91	351.93	>1000	245.93	13.29	575.72	>1000	279.13
HT29	558.75	>1000	>1000	386.73	44.60	827.66	>1000	180.82
MCF7	5.23	131.30	>1000	133.16	2.04	394.94	>1000	189.45
C6	15.36	70.61	760.55	121.48	11.71	103.01	>1000	157.97
Vero	79.85	>1000	>1000	214.99	41.43	500.42	>1000	180.74
FL	20.45	433.08	>1000	284.24	25.97	584.10	>1000	290.24

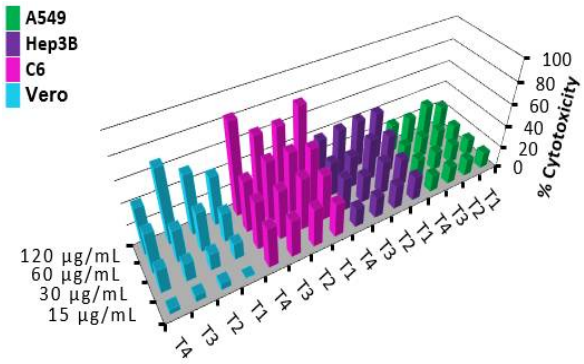
*Calculated by linear function

Lactate dehydrogenase (LDH) release

The cytotoxic effects caused by extracts in the present study was determined by measuring LDH at various concentrations (15, 30, 60 and 120 µg/mL) (Figure 1). Cyanobacterial extracts showed low cytotoxicity at all concentrations on HeLa and A549 cell lines (~10 – 20 % cytotoxicity). It has been found that at low concentrations, the cytotoxic effect of the extracts on MCF7 cell line was very low and increased along with the concentration (~5 – 40 % cytotoxicity). As in MCF7 and the Hep3B cell line, the toxicity was low at low concentrations. The extracts were found to be more toxic on other cell lines (HT29, C6, FL). *C. minutus and A. oryzae* had low cytotoxic effects on Vero cell lines, but their cytotoxicities were high in other extracts. Compared to standard 5FU whose cytotoxicity is known to be 5 – 15 %, the extracts were found to have cytostatic effects on HeLa, A549, MCF7 and Hep3B cells. Because of their low toxicity and high antiproliferative effects, the extracts examined in the present study are suitable for drug development and they merit more advanced pharmacological investigations.



A



B

Figure 1: The cytotoxic activities of cyanobacterial extracts on HeLa, HT29, MCF7 and FL (***A***), and A549, Hep3B, C6 and Vero (***B***) cells. T1: *Chroococus minutus,* T2: *Geitlerinema carotinosum,* T3: *Nostoc linckia,* T4: *Anabaena oryzae*

Morphological correlation with cytotoxic activity

Morphological changes caused by high anticancer activity of the *G. carotinosum* extract on C6 cell and low cytotoxicity on the HeLa cell were examined by inverse phase-contrast microscopy (Figure 2).

.

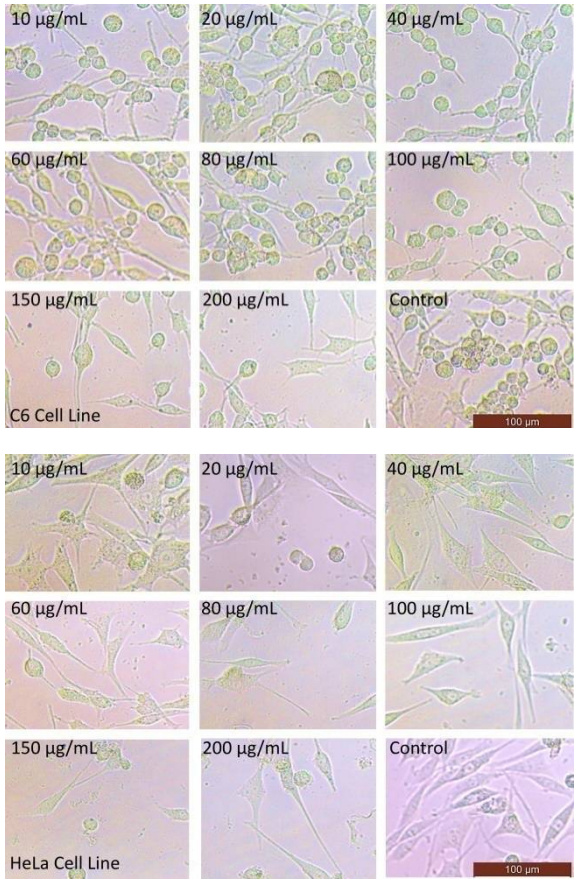


Figure 2: Effect of *G. carotinosum* extract on morphology of C6 and HeLa cells. DMSO-treated cells are controls

DISCUSSION

Cyanobacteria are oxygen producing organisms and they can live almost everywhere. They are a source of rich bioactive natural products [16]. The medical value of cyanobacteria has been known for a long time. Indeed, some cyanobacteria have been used for the treatment of some illnesses such as gout, fistula and various types of cancer [17,18]. Aqueous extract of *Spirulina platensis* was reported to inhibit growth of Human Pancreatic Adenocarcinoma (PA-TU-8902) [19]. Similarly, *Phormidium* sp. was shown to inhibit the growth of HT29 and HeLa cells [20,21].

Due to the increasing cancer incidence in the world and high level of side effects by commonly

used chemotherapy drugs, there has been an interest in use of natural products in cancer treatment recently. In the present study, antiproliferation and cytotoxicity features of cyanobacteria were examined on various cell lines to reveal their anticancer potential. Of the cyanobacteria studied, *G. carotinosum* showed the highest anticancer activity. It has been reported that anticancer effects of single filament cyanobacteria are high [22]. Similarly, *Geitlerinema* sp. CCC728 and *Arthrospira* sp. CCC729 were considered to be potent anticancer drug sources [9]. In addition, the LC_{50} is important in cancer research and the fact that the LC50 is low indicates the high activity of cancer cells [23].

LDH assay in culture medium is used as an indicator for cytotoxicity [24]. Lactate dehydrogenase is a cytoplasmic enzyme found in all cells. When cells are exposed to toxic effects, plasma membrane integrity is impaired and LDH enzyme penetrates into cells. Thus, cell damage can be assessed by measuring LDH activity after exposure to toxic effects [25]. In the present study, some of the cyanobacterial extracts displayed considerable antiproliferative activities on various cancer cell lines, and they did not have any cytotoxicity on FL (human amnion cells) and Vero (kidney epithelial cell) cells. Thus, these extracts have the potential to be used in pharmacology.

Apoptosis leads to characteristic changes (morphology) and death of cells [26]. Cyanobacterial extracts have been found to contain long acting apoptotic compounds [10]. Research demonstrated that *Oscillatoria terebriformis* extract reduced cell viability of Human Lung Cancer Cell Lines by induction of apoptosis [27]. It was observed that *Spirulina* sp. extract enriched with selenium inhibited the growth of breast cancer cells via induction of apoptosis [28]. Their ease of culturing and promising anticancer activities make cyanobacteria particularly attractive organisms for drug development through future biotechnological studies.

CONCLUSION

G. carotinosum, N. linckia and A. oryzae display considerable activity against various cancer cells. Therefore, these cyanobacteria merit investigation as part of a drug discovery process. The bioactive secondary metabolites should be isolated and identified from corresponding cyanobacteria to determine activity-compound interactions.

DECLARATIONS

Acknowledgement

The author is grateful to Dr Ramazan ERENLER for providing laboratory facilities and also to Dr Nejdet Kandemir for editing the manuscript.

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

The authors declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by them.

REFERENCES

1. *Berman-Frank I, Lundgren P, Chen Y-B, Küpper H, Kolber Z, Bergman B, Falkowski P. Segregation of nitrogen fixation and oxygenic photosynthesis in the marine cyanobacterium Trichodesmium. Sci 2001; 294(5546): 1534-1537.*
2. *Lee RE, Phycology. Cambridge University Press, 2008.*
3. *Nagarajan M, Maruthanayagam V, Sundararaman M. A review of pharmacological and toxicological potentials of marine cyanobacterial metabolites. J Apply Toxicol 2012; 32(3): 153-185.*
4. *Tan LT. Pharmaceutical agents from filamentous marine cyanobacteria. Drug discovery today 2013; 18(17): 863-871.*
5. *Costa M, Costa-Rodrigues J, Fernandes MH, Barros P, Vasconcelos V, Martins R. Marine cyanobacteria compounds with anticancer properties: A review on the implication of apoptosis. Marine drugs 2012; 10(10): 2181-2207.*
6. *Martins RF, Ramos MF, Herfindal L, Sousa JA, Skærven K, Vasconcelos VM. Antimicrobial and cytotoxic assessment of marine cyanobacteria-Synechocystis and Synechococcus. Marine drugs 2008; 6(1): 1-11.*
7. *Tan LT. Filamentous tropical marine cyanobacteria: a rich source of natural products for anticancer drug discovery. J Apply Toxicol 2010; 22(5): 659-676.*
8. *Shanab SM, Mostafa SS, Shalaby EA, Mahmoud GI. Aqueous extracts of microalgae exhibit antioxidant and anticancer activities. Asian Pac J Trop Biomed 2012; 2(8): 608-615.*
9. *Srivastava A, Tiwari R, Srivastava V, Singh TB, Asthana RK. Fresh water cyanobacteria Geitlerinema sp. CCC728 and Arthrospira sp. CCC729 as an anticancer drug resource. PloS one 2015; 10(9): e0136838.*
10. *Oftedal L, Skjærven KH, Coyne RT, Edvardsen B, Rohrlack T, Skulberg OM, Døskeland SO, Herfindal L. The apoptosis-inducing activity towards leukemia and lymphoma cells in a cyanobacterial culture collection is*

not associated with mouse bioassay toxicity. J Ind Microbiol Biotechnol 2011; 38(4): 489-501.
11. Karan T, Erenler R, Altuner Z. Isolation and Molecular Identification of Some Blue-Green Algae (Cyanobacteria) from Freshwater Sites in Tokat Province of Turkey. Turk J Agricult Food Sci Technol 2017; 5(11): 1371-1378.
12. El Sadek DAA, Hamouda RA, Bassiouny K, Elharoun H. In vitro Antioxidant and Anticancer Activity of Cyanobacteria. Asian J Med Health 2017; 6(3): 1-9.
13. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J Immunol Methods 1983; 65(1-2): 55-63.
14. Karan T, Yildiz I, Aydin A, Erenler R. Inhibition of Various Cancer Cells Proliferation of Bornyl Acetate and Essential Oil from Inula graveolens (Linnaeus) Desf. Rec Nat Prod 2018; 12(3): 273-283.
15. Suja M, Vasuki S, Sajitha N. Anticancer activity of compounds isolated from marine endophytic fungus Aspergillus terreus. World J Pharm Pharm Sci 2014; 3: 661-672.
16. Sivonen K, Börner T. Bioactive compounds produced by cyanobacteria. The cyanobacteria: molecular biology, genomics and evolution 2008: 159-197.
17. Burja AM, Banaigs B, Abou-Mansour E, Burgess JG, Wright PC. Marine cyanobacteria—a prolific source of natural products. Tetrahedron 2001; 57(46): 9347-9377.
18. Shalaby E. Algae as promising organisms for environment and health. Plant Signal Behav 2011; 6(9): 1338-1350.
19. Koníčková R, Vaňková K, Vaníková J, Vánňová K, Muchová L, Subhanová I, Zadinová M, Zelenka J, Dvořák A, Kolárř M. Anti-cancer effects of blue-green alga Spirulina platensis, a natural source of bilirubin-like tetrapyrrolic compounds. Annals of Hepatology 2014; 13(2): 273-283.
20. Salvador LA, Paul VJ, Luesch H. Caylobolide B, a macrolactone from symplostatin 1-producing marine cyanobacteria Phormidium spp. from Florida. J Nat Prod 2010; 73(9): 1606-1609.
21. Tokur O, Aksoy A. In Vitro Sitotoksisite Testleri. Harran Univ Vet Fak Derg 2017; 6(1); 112-118.
22. Costa M, Garcia M, Costa-Rodrigues J, Costa MS, Ribeiro MJ, Fernandes MH, Barros P, Barreiro A, Vasconcelos V, Martins R. Exploring bioactive properties of marine cyanobacteria isolated from the Portuguese coast: high potential as a source of anticancer compounds. Marine drugs 2013; 12(1): 98-114.
23. Al-Saadi MS, Rostom SA, Faid-Allah HM. In vitro antitumor screening of some polysubstituted pyrazole analogs. Saudi Pharm J 2005; 13: 89-96.
24. Johnson JJ, Mukhtar H. Curcumin for chemoprevention of colon cancer. Cancer letters 2007; 255(2): 170-181.
25. Niles AL, Moravec RA, Hesselberth PE, Scurria MA, Daily WJ, Riss TL. A homogeneous assay to measure live and dead cells in the same sample by detecting different protease markers. Anal Biochem 2007; 366(2): 197-206.
26. Akşit H, Bildik A. Apoptozis. YYU Vet Fak Derg 2008; 19(1): 55-63.
27. Mukund S, Sivasubramanian V. Anticancer Activity of Oscillatoria Terebriformis Cyanobacteria in Human Lung Cancer Cell Line A549. 2014.
28. Chen T, Wong Y-S. Selenocystine induces S-phase arrest and apoptosis in human breast adenocarcinoma MCF-7 cells by modulating ERK and Akt phosphorylation. J Agric Food Chem 2008; 56(22): 10574-10581.