Asian Journal of Medicine and Health

6(3): 1-9, 2017; Article no.AJMAH.34457
ISSN: 2456-8414

In vitro Antioxidant and Anticancer Activity of Cyanobacteria

Dina A. Abd El Sadek[1], Ragaa A. Hamouda[2*], Khalid Bassiouny[1] and Hala Elharoun[3]

[1]Department of Molecular Biology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City (USC), Sadat City 22857/79, Egypt.
[2]Department of Microbial Biotechnology, Genetic Engineering and Biotechnology Research Institute, University of Sadat City (USC), Sadat City 22857/79, Egypt.
[3]Department of Histology, Faculty of Medicine, Menoufia University, Egypt.

Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Article Information

DOI: 10.9734/AJMAH/2017/34457
Editor(s):
(1) Domenico Lapenna, Associate Professor of Internal Medicine, Department of Medicine and Aging Sciences, University "G. d'Annunzio" Chieti-Pescara, Chieti, Italy.
(2) Kishore Kumar Jella, Department of Radiation Oncology, Winship Cancer Institute, Emory University, Atlanta, GA, USA.
(3) Giuseppe Murdaca, Clinical Immunology Unit, Department of Internal Medicine, University of Genoa, Italy.
Reviewers:
(1) Leonel Pereira, University of Coimbra, Portugal.
(2) Christopher Larbie, Kwame Nkrumah University of Science and Technology, Ghana.
(3) Cletus Anes Ukwubile, Federal Polytechnic Bali, Nigeria.
(4) M. Lukman, Sekolah Tinggi Ilmu Farmasi Makassar, Indonesia.
Complete Peer review History: http://www.sciencedomain.org/review-history/20488

Original Research Article

Received 29th May 2017
Accepted 8th August 2017
Published 14th August 2017

ABSTRACT

Some cyanobacteria have therapeutic properties related to anticancer, antioxidant and antibacterial activity. The aim of the present study was to determine the polyphenol content together with antioxidant and anticancer activity against Ehrlich Ehrlich Ascites Carcinoma cell line *in vitro* of *Anabaena oryzae, Calothrix marchica* and *Spirulina platensis,* selecting the best alga for further investigations. The results indicate that methanol extracts of *S. platensis* has the greatest polyphenol content, together with the highest antioxidant and anticancer activity *in vitro* followed by *A. oryzae* and *C. marchica* respectively. Based on gas chromatography/mass spectrometry analysis of *S. platensis* methanol extract show that the most dominant content was octadecenoic acid methyl ester (ω- 9).

**Corresponding author: E-mail: ragaahom@yahoo.com, ragaa.hamouda@gebri.usc.edu.eg;*

Keywords: Cyanobacteria; Spirulina platensis; antioxidant; polyphenol content; Ehrlich Ascites Carcinoma cell line (EAC).

1. INTRODUCTION

Cancer is one of the most prevalent and relevant disease entity in populations of many countries worldwide [1]. Cancer represents a group of diseases characterized by uncontrolled cellular proliferation and differentiation [2]. Researches had been more attentive to natural product compounds in plants, marine organisms and microorganisms for cancer treatments [3]. Cyanobacteria are organisms that have some characteristics of bacteria and some of the algae [4]. Cyanobacteria are a prominent source of secondary metabolites [5]. Secondary metabolites play a major role in protection against insects, diseases, and many pathogens [6]. Cyanobacteria can produce many substances with anticancer, antifungal and antimicrobial effects [7]. *Anabaena oryzae* possesses bioactive compounds with antibacterial activities [8]. *Anabaena* sp and *Calothrix* sp are strains with antioxidant activities [9] *Spirulina platensis* has wide healthy benefits such as reduction in cholesterol levels, protection against allergies, and antiviral and anticancer activity [10]. *Spirulina platensis* contains ω-3 and ω-6 polyunsaturated fatty acids, provitamins and phenolic compounds [11]. Dietary supplementation with *S. platensis* is helpful in the prevention and treatment of atherosclerosis, diabetes, and cancer [12]. Dillon et al. [13] reported that *Spirulina platensis* is important for human nutrition due to the presence of polyunsaturated fatty, (ω-3 and ω-6), provitamins and phenolic compounds. *Spirulina platensis* is non toxic and has no side effects [14]. Cyanobacteria can produce secondary metabolites that have been shown to possess antibacterial, antibacterial, antifungal and anticancer activity [15]. Cyanobacteria have cytotoxic effects against eight cancer cell lines [16].

The aim of the present study was to investigate the antioxidant and anticancer activity of blue green algae, evaluating also their phenolic content and chemical composition.

2. METHODOLOGY

2.1 Algae

The algae *A. oryzae, C. marchica and S. platensis* were taken from microbiology laboratory, Genetic Engineering and Biotechnology Research Institute (GEBRI), University of Sadat City, Sadat City Egypt.

2.2 Cultivation of Algae

The Cyanobacteria *A. oryzae* and *C. marchica* were grown in 500 mL Erlenmeyer flasks containing 200 mLof BG11 medium. *S. platensis* was grown in Erlenmeyer flasks containing 200 mL Zarrouk's medium. The flasks were incubated under natural day and night at 25±1℃ for 20 days. Then algae were centrifuged, washing with distilled water three times and dried at 70℃ until constant weight [17].

2.3 Algae Extraction

Dry algae were soaked three times with 100 mL of methanol for 48hrs. After filtration methanol was evaporated and kept in freezers until use.

2.4 Evaluation of Antioxidant Activity *In vitro* by Determination of the Scavenging Capacity against the Stable Free Radical 2,2-diphenyl-1-picrylhydrazyl (DPPH)

Different concentration of algal extract (0.20, 0.40 0.60, 0.80 and 1.0 mg/mL) were tested for antioxidant activity by the DPPH method. One mL of 0.003 g DPPH in 50 mL methanol were mixed to 1.0 mL algal extracts and incubated for 30 minutes at room temperature, the DPPH-related absorbance values were determined spectrophotometrically at 517 nm using an Unico UV-2000 spectrophotometer. The antioxidant capacity was calculated according to the following equation:

$$\text{Antioxidant activity \%} = \frac{B-S}{B} * 100$$

(S) Absorbance of sample and (B) absorbance of Blank.

2.5 Total Phenolic Content (TPHC)

A different concentration of methanol extract was examined for TPHC according to the method described by Taga et al. [18] methods. One mL of methanol extracts was mixed with 750 µl of Folin Ciocalteau's (diluted ten times) and allowed to stand for 5 then, 750 µl of 6% Na_2CO_3 was added, followed by standing in the dark for 90

min at room temperature. TPHC was measured spectrophotometrically at 720 nm. The concentration was determined using tannic acid standard curve.

2.6 Assessment of Antitumor Activities against EAC *In vitro*

Ehrlich Ascites Carcinoma (EAC) was purchased from the National Cancer Institute, Cairo University, Egypt. Different concentrations of methanol extracts of *A. oryzae, C. marchica* and *S. platensis* were examined for antitumor activities against (EAC) by trypan blue method of Freshney, [19].

The percentage of non-viable cells was calculated according to the following equation:

Percentage of reduction = (non-viable cells x100) / total cells

2.7 Gas Chromatography–Mass Spectrometry (GC/MS)

Methanol extracts *S. platensis* were analysed according to El Sheekh and Hamouda [20].

2.8 Statistical Analysis

Results were expressed as means ± SEM. Statistical analysis was perfored by ANOVA plus Duncan's multiple range test. Significant differences between the means of parameters (LSD) were carried out using SPSS software.

3. RESULTS AND DISCUSSION

3.1 Antioxidant Activity *In vitro* Assessed by the DPPH Method

Fig. 1 denoted that the blue green algae had antioxidant activity. Methanol extracts of cyanophyta (*A. oryzae* and *C. marchica* and *S. platensis*) possessed the ability to scavenge DPPH substantially in a concentration-dependent fashion. *S. platensis* methanol extracts had the most efficient scavenging capacity, followed by methanol extracts of *A. oryzae* and *C. marchica,* respectively. The highest concentrations of methanol extracts of algae possessed the highest scavenging activity against DPPH. The results indicated that the radical scavenging ability of *S. platensis* methanol extracts may result in significant antioxidant effects. *S. platensis* biomass had high antioxidant activity supporting its use as nutritional supplement [21]. *S. platensis* water extracts had high antioxidant capacity [22]. Results indicated that methanol extracts of *A. oryzae* had antioxidant activity up to 36% according to concentrations of extract. Methanolic extract of the *Anabaena variabilis* caused reductions of DPPH radical scavenging activity at 16% [23]. *Spirulina* biomass contains phycobiliproteins, phycocyanin and alophycocyanin, which have antioxidant properties [24].

3.2 TPHC of Blue Green Algae

Phenolic contents have antioxidant properties and relevant biological characteristics. Phenolic compounds such as tannic, rutin and gallic acid gave high antioxidant capacity [25]. The results of Figs. 1 and 2 indicated that there is basically a positive relationship between antioxidant activity and TPHC. In particular, the highest TPHC of *Spirulina platensis* was related to its highest antiradical-antioxidant capacity. *S. platensis* had more phenolic content, followed by *A. oryzae* and *Calothrix marchica,* respectively. The highest TPHC of *Spirulina platensis* related to the algal samples rich in antioxidant capacity [26]. Islam et al. [27] reported that phenolic compounds found in algae have various health-promoting functions in humans. *Spirulina platensis* contained significant amounts of phenolics compounds [28]. Wu et al. [29] indicated that *Spirulina* had five times higher amounts of phenolic contents compared to *Chlorella. Spirulina platensis* deserves special attention due to its importance as human food and the associated *in vitro* and/or *in vivo* antioxidant potential [13].

3.3 Antitumor Activities against Ehrlich Ascites Carcinoma (EAC) *In vitro*

Various concentrations (200, 400, 600, 800, 1000 µg/mL) of methanol extracts of *S. platensis, A. oryzae* and *C. marchica* were investigated against EAC *in vitro*. The results in Table 1 and Fig. 3 indicated that *S. platensis, A. oryzae* and *C. marchica* inhibited EAC proliferation. The *S. platensis* showed more antiproliferative effects against EAC than *A. oryzae* and *C. marchica,* respectively. The maximum percentage of EAC inhibition was 89%, 69%, and 61%, respectively, with 1000 µg/mL *S. platensis*, *A. oryzae* and *C. marchica*. Doleyres and Lacroix [30] reported that cyanobacteria have provided beneficial physiological effects on human health, such

as antitumor activity, immune modulating bioactivity and antimutagen capacity. Mirada et al. [31] reported that some types of cancer were inhibited by algal extracts. Abu Zaid et al. [22] showed that water extracts of *S. platensis* had antiproliferative properties in human colon carcinoma cells and hepatocellular carcinoma cells.

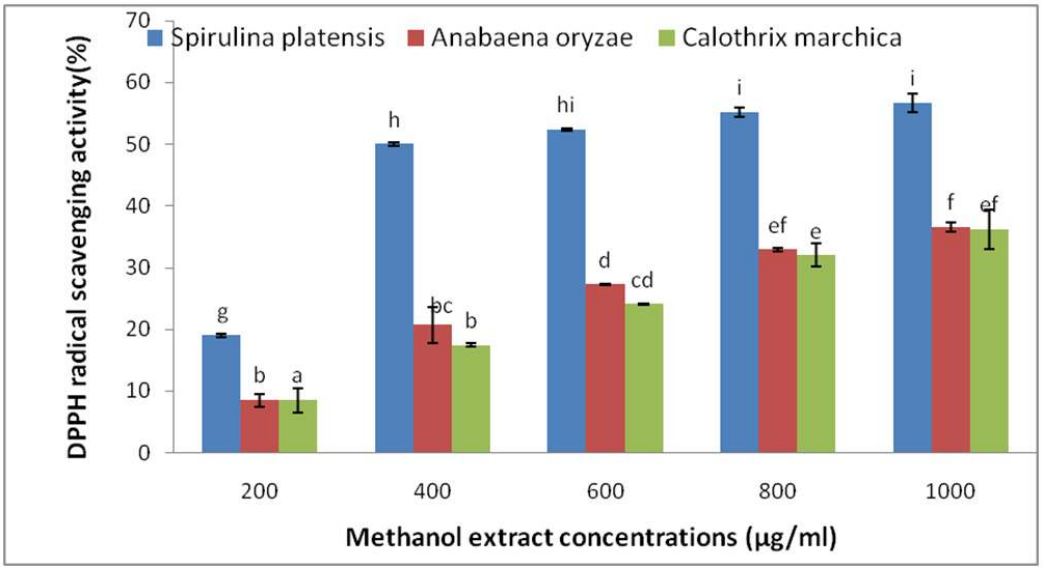


Fig. 1. Antioxidant activity of methanol extracts (µg/mL^{-1}) of blue green algae (*S. platensis*, *A. oryzae* and *C. marchica*) determined by DPPH

The same letter(s) are not significantly different according to Duncan's multiple range test Bars represent means ± SEM of three independent experiments

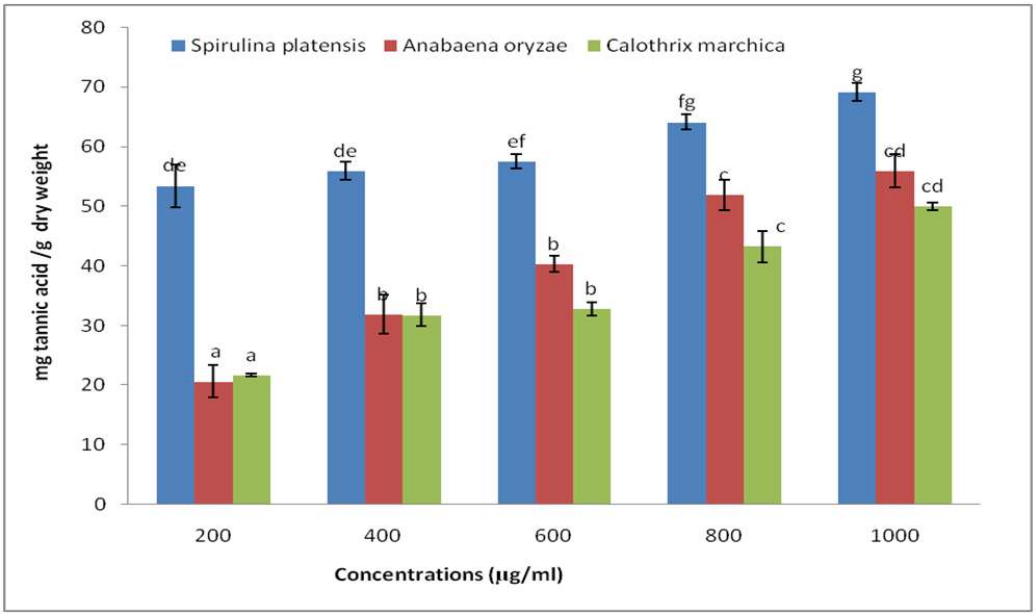


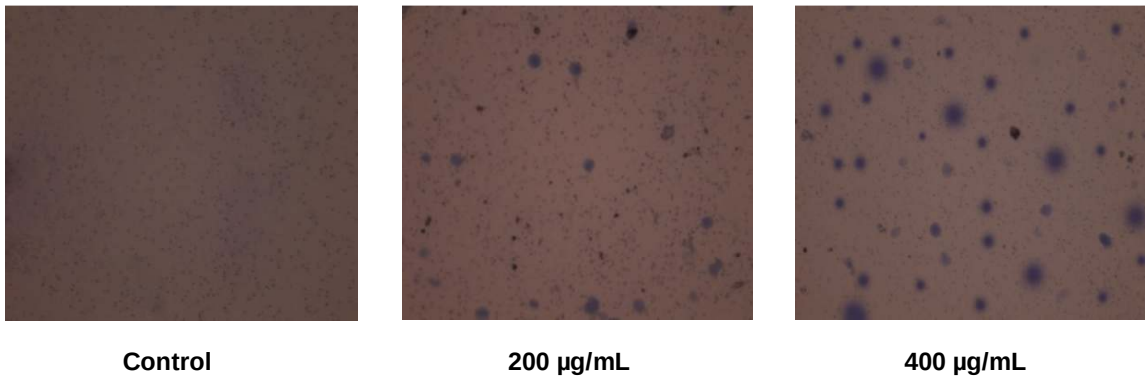
Fig. 2. Total phenolic in different concentrations of methanol extracts (µg/mL) of tested blue green algae (*S. platensis*, *A. oryzae* and *C. marchica*). Bars are standard error

The same letter(s) are not significantly different according to Duncan's multiple range tests. Bars represent means ± SEM of three independent experiments

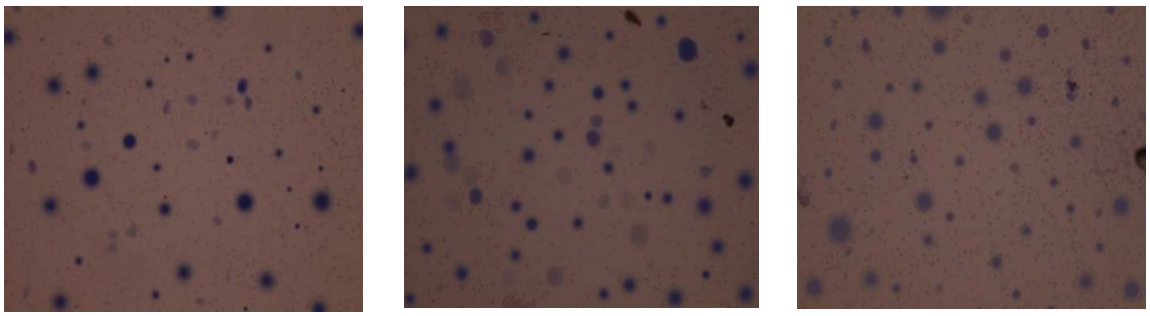
Table 1. *In vitro* cytotoxic effect of different methanol extracts concentrations of *Anabaena oryzae, Calothrix marchica*, and *Spirulina platensis* on the viability of EAC

Concentrations µg/ml	*Anabaena oryzae*	*Calothrix marchica*	*Spirulina platensis*
	Percentage of reduction (non viable cells)		
200	4±0 a	3±0.75a	5±0.75a
400	20±0.57b	18±0.57b	25±0.57c
600	35±1 d	28±1.15e	40±1.15f
800	60±1.15 g	55±4.48h	71±1.15i
1000	69±2.33h	61±1.7i	89±1.45j

Data represent means ± SEM of three independent experiments. The same letter(s) are not significantly different according to Duncan's multiple range test

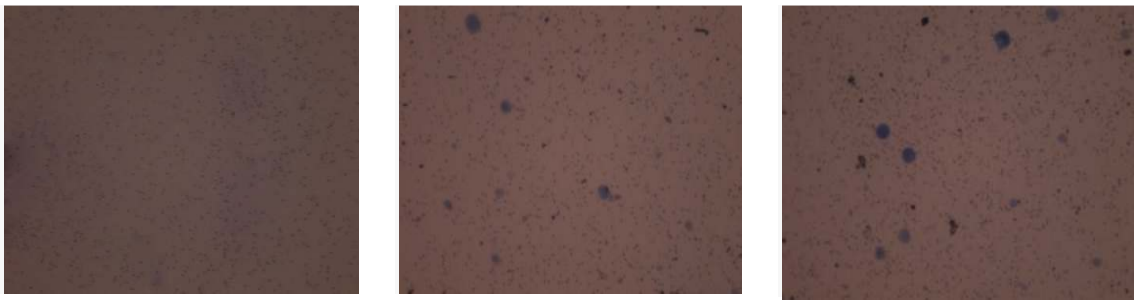


Control **200 µg/mL** **400 µg/mL**

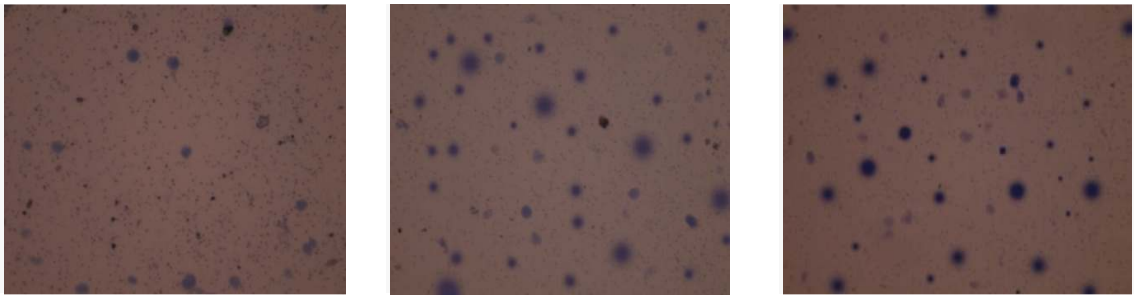


600 µg/mL **800 µg/mL** **1000 µg/mL**

Fig. 3a. Cytotoxic effect of *Spirulina platensis* methanol extracts against EAC cells

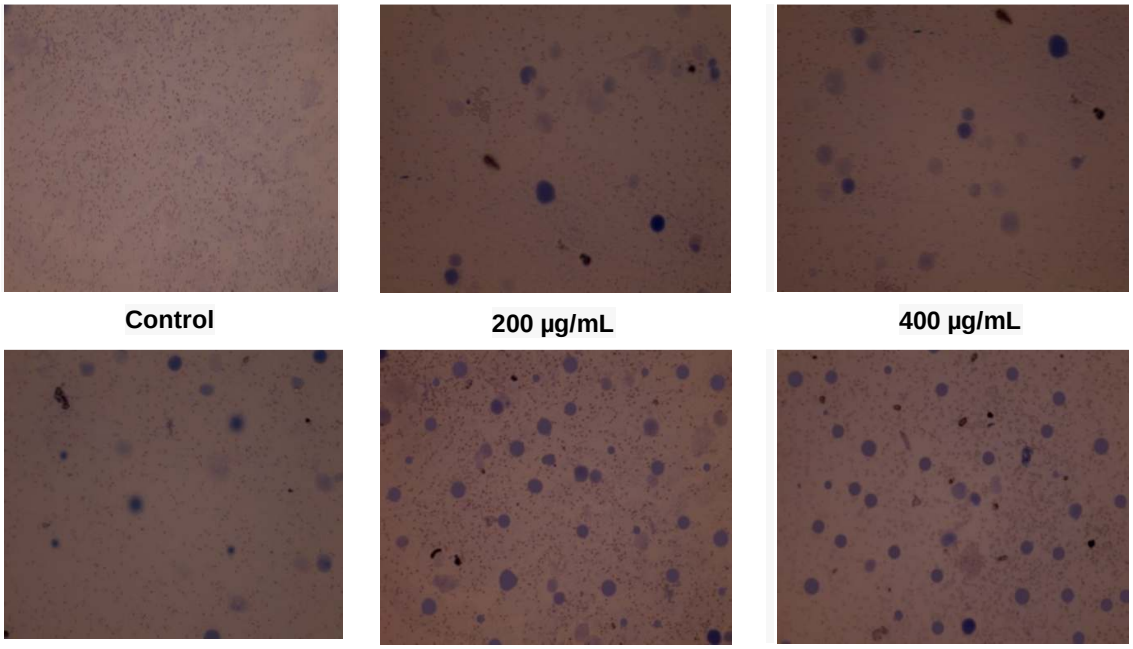


Control **200 µg/mL** **400 µg/mL**



600 µg/mL 800 µg/mL 1000 µg/mL

Fig. 3b. Cytotoxic effect of *Anabaena oryzae* methanol extracts against EAC cells



Control 200 µg/mL 400 µg/mL

600 µg/mL 800 µg/mL 1000 µg/mL

Fig. 3c. Cytotoxic effect of *Calothrix marchica* methanol extracts against EAC cells

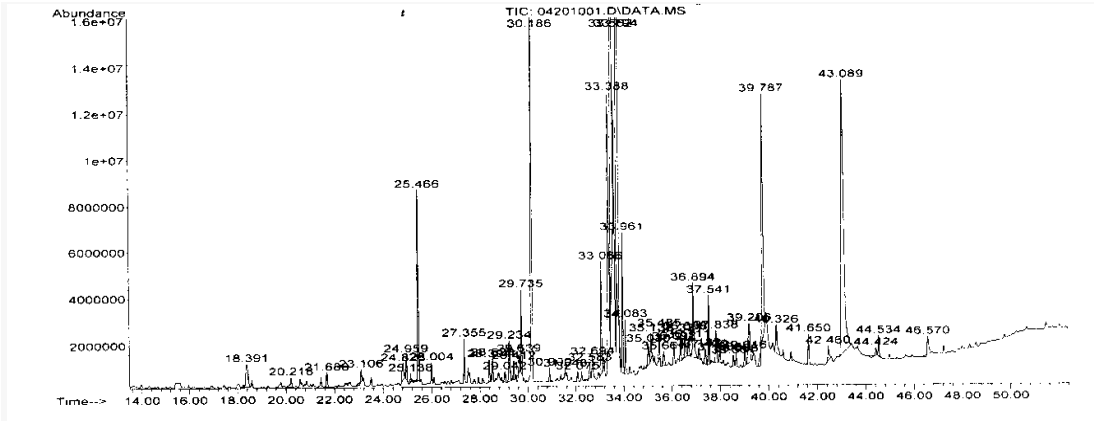


Fig. 4. GC/MS profiles of *Spirulina platensis* methanol extracts

Table 2. Major chemical composition of *S. platensis* methanol extracts

Compounds	Chemical formula	Retention time (R.T)	Area%
Heptadecane	$CH_3(CH2)_{15}CH_3$	25.468	3.83
Hexadecene	$CH_3(CH2)_{13}CH{=}CH_2$	29.233	1.03
Hexadecenoic acids methyl ester	$C_{17}H_{34}O_2$	29.734,30.184	11.76
Gamma linolenic acids methyl ester	$C_{19}H_{32}O_2$	33.070	2.43
8,11 octadecadienoic acid methyl ester (omega 6)	$C_{19}H_{34}O_2$	33.384	6.11
Octadecenoic acid methyl ester Oleic acids(omega 9)	$C_{19}H_{34}O_2$	33.562	13.81
2-Hexadecenol	$C_{16}H_{34}O$	33.792	13.39
Octadecanoic methyl ester	$C_{19}H_{34}O_2$	33.961	2.68
Cyclopentanone	C_5H_8O	34.080	1.07
2(3H)-Furanone	$C_4H_6O_3$	36.890	2.15
Octadecenoic acid methyl ester Oleic acids(omega 9)	$C_{19}H_{36}O_2$	39.785	6.6
9-Octadecenoic acid	$C_{18}H_{34}O_2$	43.087	13.8

3.4 GC/MS Analysis of *Spirulina platensis* Methanol Extracts

Table 2 and Fig. 4 denote that there are thirteen major components in the chemical composition of methanol extracts of *S. platensis*. Chemical components are represented by octadecenoic acid methyl ester (ω- 9), 9-octadecenoic acid, 2-hexadecenol, hexadecenoic acids methyl ester, 8,11 octadecadienoic acid methyl ester (omega 6), heptadecane, octadecanoic methyl ester, gamma linolenic acids methyl ester, 2(3H)-furanone and cyclopentanone. One of the major component of *S. platensis* was heptadecane that possesses antioxidant properties [32,33]. Percentage area of hexadecene was 1.03 at retention time 29.233. Kumar et al. [34] reported that acetone extracts of *Spirulina platensis* contain hexadecene and had antibacterial activity. The area of hexadecenoic acids methyl ester is 11.76 of *S. platensis* methanol extracts that possesses antibacterial, antioxidants and antitumour properties [35]. Gamma linolenic acids methyl ester extracted from *S. platensis* had cytotoxic activity against human lung carcinoma cell lines [36]. *S. platensis* also contains unsaturated fats such as omega 6 and omega 9 which play a noticeable role in human health [37].

4. CONCLUSION

S. platensis is the best alga with anticancer activity against EAC *in vitro*. There are thirteen major compounds present in *S. platensis* methanol extracts according to GC/MS analysis. The highest content of alga was octadecenoic acid methyl ester (oleic acids, ω- 9) which represents 20.41% of total content of methanol extract.

CONSENT

It is not applicable.

ETHICAL APPROVAL

It is not applicable.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

REFERENCES

1. Jamal S. Mamoon N. Mushtaq S. Luqman M. Carcinoma of the male breast: A study of 141 cases from Northern Pakistan. Asian Pac J Cancer Prev. 2006;7:119-21.
2. Ponder BAJ. Cancer genetics. Nature. 2001;411:336-341.
3. Zandi K, Ahmadzadeh S, Tajbakhsh S. Anticancer activity of *Sargassum oligocystum* water extract against human cancer cell lines. Eur Rev Med Pharmacol Sci. 2010;14:669–673.
4. Oshima Y. Hirota M. Yasumoto T. Hallegraeff G. Blackburn S. Steffensen D. Production of paralytic shellfish toxins by the dinoflagellate *Alexandrium minutum* Halim from Australia. Nipp. Suis. Gakk. 1989;55:925.
5. Jones AC, Monroe EA, Eisman EB, Gerwick L, Sherman DH, Gerwick WH.

The unique mechanistic transformations involved in the biosynthesis of modular natural products from marine Cyanobacteria. Natural Product Reports. 2010;27:1048–1065.
6. Wink M. plant breeding importance of plant secondary metabolites for protection against pathogens and behaviors. Theor. Appl. Genet. 1988;75:225-233.
7. Volk RB, Furkert FH. Antialgal antibacterial and antifungal activity of two metabolites produced and excreted by Cyanobacteria during growth. Microbiol. Res. 2006;161: 180–186.
8. Digamber RB. Antibacterial activity of fresh water Cyanobacteria. J. Algal Biomass Utln. 2015;6(3):60-64.
9. Kumar R, Elumalai S, Pabbi S. Evaluation of antioxidant activity and total phenolic content in Cyanobacteria isolated from different regions of India. International Journal of Scientific Research. 2015;4:17-20.
10. Konícková R, Vnková K, Vaníková J, Vánová K, Muchová L, Subhanová I, Zadinová M, Zelenka J, Dvorák A, Kolár M, et al. Anti-cancer effects of blue-green alga *Spirulina platensis*, a natural source of bilirubin-like tetrapyrrolic compounds. Ann. Hepatol. 2014;13:273–283.
11. Pascaud M. The essential polyunsaturated fatty acids of *Spirulina* and our immune response. Bulletin de l, Institute Oceanographique. 1993;12:49-57.
12. Gershwin ME. Belay A. *Spirulina* in human nutrition and health. Boca Raton: CRC Press; 2008.
13. Dillon JC. Phan PA. *Spirulina* as source of protein in human nutrition. Bulletin de l'Institute Oceanographique. 1993;12:103-107.
14. Desai K, Sivakami S. *Spirulina*: the womder food of the 21th century. Asia Pac Biotech News. 2004;8:1298-1302.
15. Jimenez JI, Vansach T, Yoshida WY, Sakamoto B, Porzgen P, Horgen FD. Halogenated fatty acid amides and cyclic depsipeptides from an eastern Caribbean collection of the cyanobacterium *Lyngbya majuscula*. J. Journal of Natural Products. 2009; 72:1573–1578.
16. Costa GM, Rodrigues JC, Costa SM, Ribeiro JM, Fernandes MH. Barros P. Barreiro A. Vitor VV, Martins R. Exploring bioactive properties of marine Cyanobacteria isolated from the Portuguese coast: High potential as a source of anticancer compounds. Mar. Drugs. 2014;12:98-114.
DOI: 10.3390/md12010098
17. Khalifa KS, Hamouda RA, Hamza HA. Antitumor activity of silver nanoparticles biosynthesized by micro algae. Journal of Chemical and Pharmaceutical Research. 2016;8(3):1-6.
18. Taga MS. Miller EE. Pratt DE. Chia seeds as a source of natural lipid antioxidants. J. Am. Oil Chem. Soc. 1984;61:928-931.
19. Freshney RI. Culture of animal cells. A manual of basic techniques; 3rd ed. New York Wiley-Liss; 1994.
20. El-Sheekh MM, Hamouda RA. Lipids extraction from the green alga *Ankistrodesmus falcatus* using different methods. Rendiconti Lincei. Scienze Fisiche e Naturali. 2016;27:589–595.
21. Colla LM, Furlong EB, Costa JAC. Antioxidant properties of *Spirulina* (Arthospira) *platensis* cultivated under different temperatures and Nitrogen Regimes. Brazilian Archives of Biology and Technology. 2007;50:161-167.
22. Abu Zaid AA. Hammad DM. Sharaf EM. Antioxidant and anticancer activity of *Spirulina platensis* water extracts. International Journal of Pharmacology. 2015;11:846-851.
23. Pant G. Kumar G. Karthik L. Prasuna RG. Rao KVB. Antioxidant activity of methanolic extract of blue green algae *Anabaena* sp. (Nostocaceae) European Journal of Experimental Biology. 2011;1: 156-162.
24. Estrada JE. Bescós P. Villar DAM. Antioxidant activity of different fractions of *Spirulina platensis* protean extract. *IlFarmaco*. 2001;56:497-500.
25. El Salhin H. Abobaker HM. Hasan AHM. El-Dayek JEE. Antioxidant capacity and Total phenolic compounds of some algae species (*Anabaena* and *Spirulina platensis*). Scholars Academic Journal of Biosciences (SAJB). 2016;4(10A):782-786.
26. Machu L, Misurcova L, Ambrozova JV, Orsavova J. MLcek J. Sochor J. Jurikova T. Phenolic content and antioxidant capacity in algal food products. Molecules. 2015;20:1118-1133.
DOI: 10.3390/molecules20011118
27. Islam MS, Yoshimoto M, Yamakawa O. Distribution and physiological functions of caffeoyl quinic acid derivatives in leaves of sweet potato genotypes. Journal Food Science. 2003;68:111-116.

28. Halimoon N, Ali R. Evaluation of antioxidative activity of phenolics in methanolic extracts of blue green algae. Catrina. 2010;5(1):9-13.
29. Wu LC, HO JA, Shieh MC, Lu IW. Antioxidant and antiproliferative activities of *Spirulina* and *Chlorella* water extracts. Journal of Agricultural and Food Chemistry. 2005;53(10):4207-4212.
30. Doleyres Y. Lacroix C. Technologies with free and immobilised cells for probiotic bifidobacteria production and protection. Int. Dairy J. 2005;15:973-988.
31. Mirada MS. Cintra RG. Barros SB. Mancini-Filho J. Antioxidant activity of the microalga *Spirulina maxima*. Brazailian Journal of Medical and Biological Rese arch. 1998;31(8):1075-1079.
32. Ozdemir G, Karabay NU, Dalay MC, Pazarbasi B. Antibacterial activity of volatile component and various extracts of *Spirulina platensis*. Phytother Res. 2004; 18:754–757 .
33. Kim DH, Park MH, Choi YJ, Chung KW, Park CH, Jang EJ, et al. Molecular study of dietary heptadecane for the anti-inflammatory modulation of NF-kB in the aged kidney. PLoS ONE. 2013;8(3): e59316.
34. Kumar V, Bhatnagar AK, Srivastava JN. Antibacterial activity of crude extracts of *Spirulina platensis* and its structural elucidation of bioactive compound. Journal of Medicinal Plants Research. 2011;5(32): 7043-7048.
35. Govindappa M, Channabasava R, Sadananda TS, Chandrappa CP, Umashankar T. Identification of bioactive metabolites by GC-MS from an endophytic fungus, alternaria alternate from tabebuia argentea and thrir *in vitro* cytotoxic activity. International Journal of Biological & Pharmaceutical Research. 2014;5(6):527-534.
36. Jubie S. Dhanabal SP. Chaitanya MV Isolation of methyl gamma linolenate from *Spirulina platensis* using flash chromatography and its apoptosis inducing effect. BMC Complement Altern Med. 2015;4:15-263.
37. Otles S. Pire R. Fatty acid composition of *Chlorella* and *Spirulina* microalgae species. JAOAC Int. 2001;84(6):1708-14.

Peer-review history:
The peer review history for this paper can be accessed here:
http://sciencedomain.org/review-history/20488