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In vitro Anticancer Potentialities of Three Egyptian Cyanobacterial Isolates against Breast Adenocarcinoma and Hepatocellular Carcinoma Cell Lines

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CYANOBACTERIA represent a promising but not totally explored source of a wide array of secondary metabolites. They also represent a source for the discovery of compounds and drugs. In the present study, three cyanobacterial species, namely, *Arthrospira platensis* MH285264, *Leptolyngbya boryana* MH155317, and *Leptolyngbya* sp. MH285263 were tested for their anticancer capabilities against hepatocellular carcinoma (Hep-G2) and breast adenocarcinoma (MCF-7) cell lines. In order to obtain the highest biomasses of the tested organisms, they were optimized using 8 media recipes. The optimized organisms were tested for their biochemical constituents. The GC-MS of the tested cyanobacterial extracts revealed the presence of a number of bioactive metabolites which were previously known for their anticancer activity. The extracts of *A. platensis* and *L.* sp. showed a cytotoxic effect against the Hep-G2 cell line with IC_{50} values of 14 and 15µg.mL^{-1}, respectively. The extracts of *A. platensis* and *L.* sp. showed more cytotoxic effects against the MCF-7 cell line with IC_{50} values of 12.1 and 13.2µg.mL^{-1}, respectively. On the basis of the undiluted concentration used in this study (100µg.mL^{-1}), the maximum inhibitory percentages compared with the control against the Hep-G2 and MCF-7 cell lines were 73.2% and 91.2%, respectively, by extracts of *L.* sp. and *A. platensis*. *L. boryana* exhibited a low and neglectable anticancer activity against both cell lines. The obtained promising results herald the existence of natural and safe alternatives with a high efficiency extracted from *A. platensis* and *L.* sp. for inclusion in the strategies of cancer treatment.

Keywords: Anticancer, Cell lines, Cyanobacteria, Cytotoxic, Optimization.

Introduction

Cancer is among the most serious lethal diseases known nowadays and represents the second cause of mortality globally; it was responsible for about 8.8 million deaths in 2015 (McGuire, 2016) and around 10 million deaths in 2020 (Ferlay et al., 2020). Nearly 70% of deaths due to cancer diseases are commonly recorded in low and middle-income countries (Stewart et al., 2016). Cancer is defined as a malignant neoplasm including a large number of diseases involving abnormal uncontrolled cell growth (Fior & Zilhão, 2019). In cancer, the cells undergo uncontrollable division and growth, forming a malignant tumor (Lobo et al., 2007). Hepatocellular carcinoma (HCC) is the most prevalent type of liver cancer, being the 6th commonest type of cancer and responsible for 9.1% of all cancer deaths per year; the HCC tumor is highly resistant to chemotherapeutic agents, and about 80% of the patients die within a year after diagnosis (Ahmed et al., 2017; Akbarizare et al., 2020). Meanwhile, breast cancer represents the second commonest group of cancers worldwide and the second cause of cancer deaths in women (Naaman et al., 2016).

The majority-approved method for treating cancer is based on the removal of cancerous tissue via surgery operations followed by chemotherapy and/or radiotherapy (Kranz & Dobbelstein, 2012). To date, no potent drug is available against cancer,

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and the currently available chemotherapeutic drugs are a source of numerous undesirable side effects; thus, current therapeutic strategies must be improved to find a therapeutic agent with a limited side effect (Mandal & Rath, 2015). In the recent decade, the interest on natural materials produced by plants and microorganisms for use as treatments against cancer, viral, and microbial diseases has re-emerged (Khalifa et al., 2019). Plants and microorganisms produce a wealth of natural secondary metabolites with highly antitumor efficacy and cause no effect on normal healthy cells (Senousy et al., 2020). Cyanobacteria represent a group of organisms with a unique cellular structure (Mofeed, 2019). They are a diversified and widespread group of photosynthetic prokaryotes, showing a high resemblance with gram-negative bacteria in terms of cellular organization and with green plants in terms of oxygenic photosynthesis (Kozak et al., 2019). With long evolutionary history, they are among the oldest life forms existing on earth, that is, around 3.5 billion years (Thajuddin & Subramanian, 2005). Cyanobacteria produce a large number of medicinally important secondary metabolites, such as lipopeptides, polyphenolics, alkaloids, polysaccharides, and cyclic peptides, which exhibit a wide variety of biological activities, including antimicrobial, anticancer, antiprotozoal, antioxidant, and antiviral effects (Swain et al., 2015; LewisOscar et al., 2018; Deyab et al., 2019). Cyanobacteria produce a vast number of natural products that are effective in killing cancer cells (Deyab et al., 2020). Several cyanobacteria produce compounds, such as synthadotin, cryptophycin, and curacin, which are identified to have anticancer effect and include compounds that have effectively succeeded to enter clinical trials (Singh et al., 2011; Qamar et al., 2021). Cyanobacteria isolated from marine environments are being studied extensively because 50% of these organisms are regarded as a potential source of anticancer drugs, whereas organisms isolated from freshwater habitats and soil are poorly evaluated (Agatonovic-Kustrin et al., 2018; Zhang et al., 2021). Therefore, in the present investigation, we aimed to screen the anticancer activity of three cyanobacterial extracts isolated mainly from fresh to brackish water habitats against HCC (Hep-G2) and breast cancer adenocarcinoma (MCF-7) cell lines.

Materials and Methods

Cyanobacterial isolates

Three cyanobacterial strains, namely, *Arthrospira platensis*, *Leptolyngbya boryana,* and *Leptolyngbya* sp., were used in the present study. These strains were previously isolated and identified on morphological and molecular bases (Deyab et al., 2020). The obtained sequences of these organisms were added to the GenBank database with different accession numbers as follows: *A. platensis* MH285264, *L. boryana* MH155317, and *L.* sp. MH285263. The organisms were maintained in the Phycology Laboratory, Faculty of Science, Damietta University, by serial subculturing. For obtaining the extract, the species were grown separately in a 2L Erlenmeyer flask containing 1L culture medium. Then, the flasks were incubated at 25±2°C, 1.2 Klux light intensity, and a light/dark cycle of 16:8 h for 21 days to obtain the biomass.

Optimization of Cyanobacterial culture media

To attain the best growth conditions for the tested cyanobacteria, we cultured the three species in eight media recipes, including BG11 medium (Stanier et al., 1971) and *Spirulina* media (Aiba & Ogawa, 1977) in addition to six modified media. The tested cyanobacteria were cultured in 250mL culture flasks, each containing 100mL of different media used for the optimization. All culture media were kept under the same condition for 30 days as described previously. Table 1 shows the composition of the standard BG-11 medium, *Spirulina* medium, and the six modified culture media. The dry weight (DW) and chlorophyll "a" (Chl. a) content of the harvested biomass was tested following the methods of Antal et al. (2001) and Jeffrey & Humphrey (1975), respectively.

Preparation of cyanobacterial crude extracts

The biomass of each optimized cyanobacterial species was harvested using centrifugation at 6000r/min for 10min and then frozen. After thawing, 1g of each sample was extracted twice using 10mL followed by 10mL absolute methanol (100%), following the method described by Iloki-Assanga et al. (2015), with the biomass crushed using an ultrasound sonicator at a pulse speed of 20000Hz for 10sec until all cells were broken. The extracts were kept for 24h at room temperature before centrifugation at 6000r/min for 20min to obtain the cell-free supernatant. Using the rotary evaporator, the extracts were concentrated at 40°C. The residues were redissolved in 3mL dimethyl sulfoxide and stored at 4°C until further use.

TABLE 1. Composition of different culture media used for optimization (g L^{-1})

Chemicals	BG-11	*Spirulina* media	Modified-No 1	Modified-No 2	Modified-No 3	Modified-No 4	Modified-No 5	Modified-No 6
$NaNo_3$	1.5	2.5	3	1.5	1.5	1.5	1.5	0.9
K_2HPO_4	0.04	0.04	0.04	0.08	0.02	0.08	0.04	0.02
$MgSO_4.7H_2O$	0.075	0.2	0.075	0.075	0.075	0.075	0.075	….
$CaCl_2.2H_2O$	0.036	0.02	0.036	0.036	0.036	0.036	0.036	0.036
Citric acid	0.006	0.006	0.006	0.006	0.006	0.006	0.006	0.012
Ferric ammonium citrate	0.006	….	0.006	0.006	0.006	0.006	0.006	0.002
$FeSO_4.7H_2O$	….	0.01	….	….	….	….	….	….
Na_2CO_3	0.02	4.03	0.02	0.02	0.02	0.02	0.02	….
Na EDTA	0.001	0.08	0.001	0.001	0.001	0.001	0.001	0.001
$MgCl_2$	….	….	….	….	….	….	….	0.10
$NaHCO_3$	….	13.61	….	….	….	….	….	0.05
NH_4Cl	….	….	….	0.015	….	….	….	0.026
K_2SO_4	….	1.0	….	….	….	….	….	….
Cyanocobalamin (vit. B_{12})	….	0.005	….	….	….	….	….	….
Trace Metal mix*	1ml	5ml	1ml	1ml	1ml	1ml	1ml	1ml
Final pH	7.5	9.3	7.5	7.5	7.5	7.5	4.0	7.5

*Trace metal solution (g L^{-1} dH_2O): 1.0g $ZnSO_4.7H_2O$, 2.0g $MnSO_4.7H_2O$, 10.0g H_3BO_3, 1.0g Co $(NO_3)_2.6H_2O$, 1.0g $Na_2MoO_4.2H_2O$; 0.005g $CuSO_4.5H_2O$.

Biochemical characterization

Biochemical analysis was carried out to assess the total protein, lipid, and carbohydrate contents (% of DW). The total protein content was detected by the estimation of elemental nitrogen and the use of nitrogen-protein conversion factor equal to 6.25 in accordance with the work of Cunniff & Washington (1997). The total carbohydrates were analyzed by using the method of Dubois et al. (1956) with glucose as a standard. The total lipids were estimated following the method of Cunniff & Washington (1997).

Gas chromatography-mass spectrometry (GC-MS) analysis

The chemical constituents of the cyanobacterial extracts were determined using Varian Chrompack CP-3800 GC/MS/MS-2000 equipped with split-splitless injector and DB-5.625 GC column (30m × 0.25mm i.d., 0.25µm film thickness).

Cell lines

The human HCC (Hep-G2) and breast adenocarcinoma (MCF-7) cell lines were obtained from the National Cancer Institute, Cairo University. The Hep-G2 and MCF-7 cells were cultured in Dulbecco's Modified Eagle's Medium and Roswell Park Memorial Institute-1640 medium, respectively (Sigma–Aldrich, USA). Both culture media were supplemented with antibiotic-free 10% fetal bovine serum (Sigma–Aldrich., USA), 100 $U.ml^{-1}$ penicillin, and 2mg. mL^{-1} streptomycin. The cells were incubated at 37°C in a 95% relative humidified atmosphere containing 5% CO_2.

Anticancer activity of cyanobacterial extracts using (SRB) method

The *in vitro* anticancer assay was performed using SRB assay, which represents a highly sensitive method for assessing cytotoxic activity (Skehan et al., 1990). Briefly, in 96-well microtiter plates, 3×10^3cells were seeded in a 150mL fresh medium per well and then lifted for 24h to attach to the plates in a CO_2 incubator at 37°C. On the second day, the cells were treated with the extracts of tested cyanobacteria using a broad concentration range (0, 12.5, 25, 50, and 100µg.mL^{-1}) and incubated for 48h. A fixation step was conducted by using 50µL 50% trichloroacetic acid at 4°C for 1h. The plates were then washed using distilled water and stained with 50µL 0.4% SRB dissolved in 1% acetic acid for 30min at room temperature. The excess dye was

discarded by washing it four times using 1% acetic acid. The optical density of the microplate wells was measured spectrophotometrically using an enzyme-linked immunosorbent assay reader at 570nm. The half-maximal inhibitory concentration (IC_{50}) was the concentration of cyanobacterial extract that inhibited 50% of the cancer cell lines. The percentage of relative viability was calculated using GraphPad Prism software.

Statistical analysis

All tests were performed in triplicates and presented as a mean ± standard deviation. $P < 0.01$ represented a significant difference. Statistical analysis was conducted by using SPSS 21.0 for Windows.

Results

Cyanobacterial species identity

The identity of the three cyanobacterial species was determined in a previous study (Deyab et al., 2020). To verify their identity in this study, we reconstructed a neighbor-joining phylogenetic tree using Mega 7 software (Fig. 1). A total of 550 positions were determined in the final dataset, and 180 of them were informative sites.

Optimization of cyanobacterial species

The tested cyanobacteria showed different growth rates in various media recipes used for the optimization. In general, the optimum growth of the three isolates occurred mostly on the 21st day of culture and followed by a growth decline. In spite of the good growth rates of *L. boryana*, and *L.* sp. in modified-No. 2 medium, their best growth rates were attained by the standard BG11 medium on the 21st day, with values of DW= 0.68g L^{-1} and Chl. a= 0.64mg L^{-1} for *L.* sp. (Fig. 2) and DW= 0.66g L^{-1} and Chl. a= 1.05mg L^{-1} for *L. boryana* (Fig. 3). The optimum growth of *A. platensis* was observed only on the 21st day on the *Spirulina* medium (DW= 0.95g L^{-1} and Chl. a= 1.9mg L^{-1}) (Fig. 4).

Biochemical characterization of the cyanobacterial species

Figure 5 illustrates the biochemical compositions of the three tested cyanobacterial species. The protein content fluctuated between 40.2% and 56.4% of the DW, whereas carbohydrate contents in the three species vacillated from 22.8% to 26.2%, representing about half the percentage of the protein content. Meanwhile, the total lipids ranged between 4% and 6.6% of the DW. *A. platensis* showed the highest protein, carbohydrate, and lipid contents. On the other hand, *L.* sp. recorded the lowest protein and carbohydrate contents, whereas *L. boryana* exhibited the lowest lipid contents among the tested species.

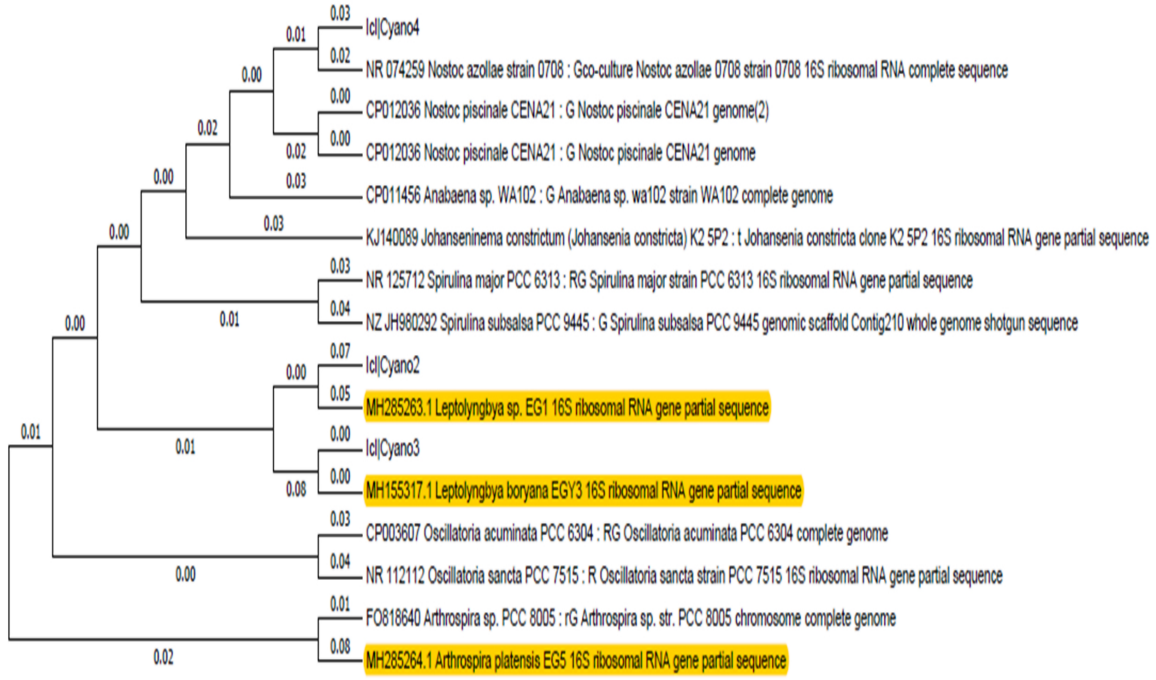

Fig. 1. Phylogenetic tree based on 16S rRNA sequences; tested stains are in bold and highlighted

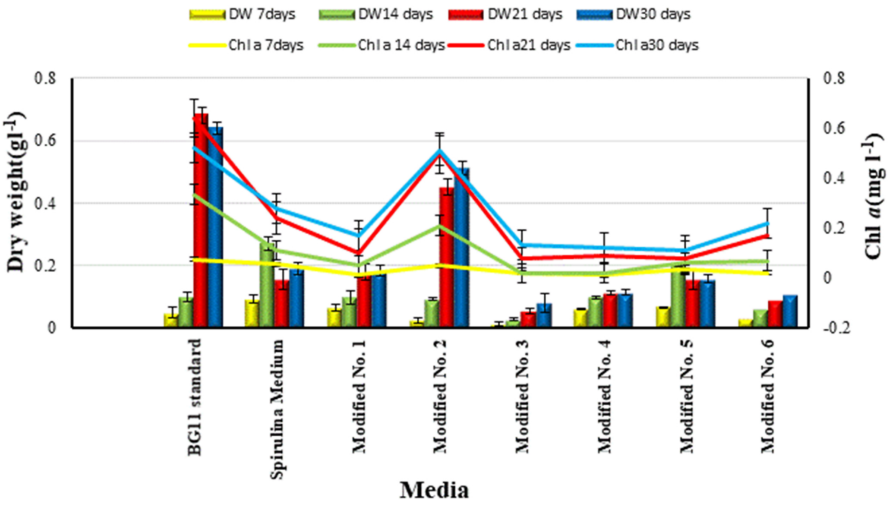

Fig. 2. Biomass estimation of *Leptolyngbya* sp. using DW and Chl. a content

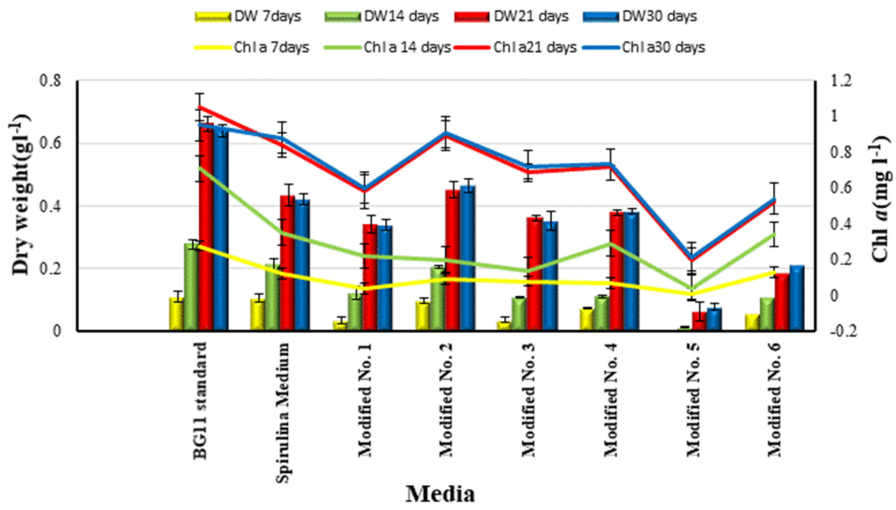

Fig. 3. Biomass estimation of *Leptolyngbya boryana* using DW and Chl.a content

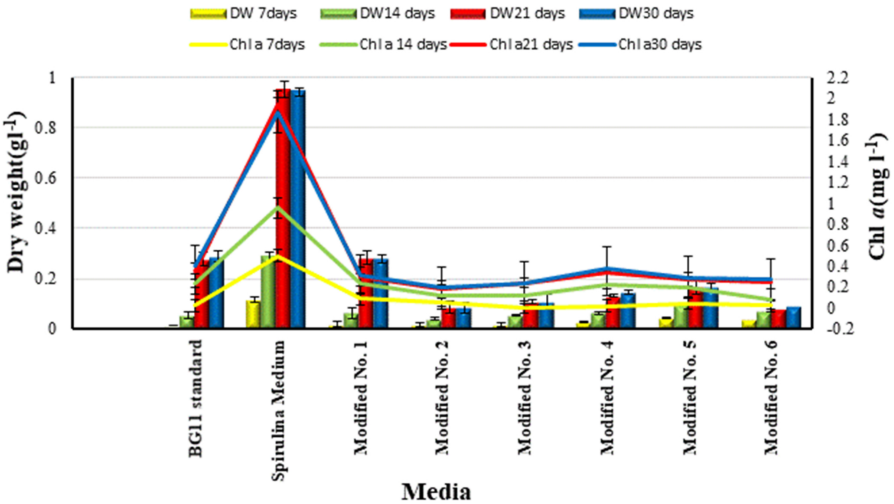

Fig. 4. Biomass estimation of *Arthrospira platensis* using DW and Chl. a content

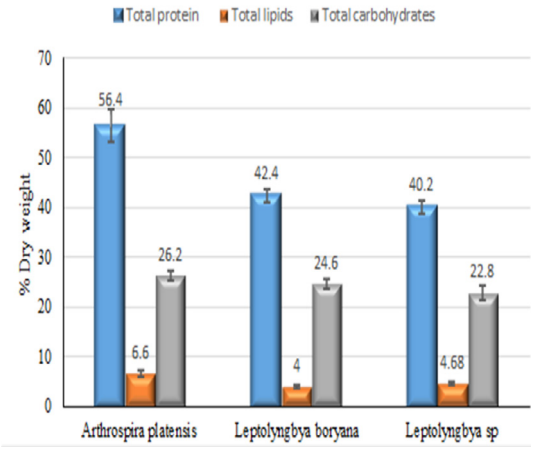

Fig. 5. Biochemical characterization (protein, lipids, and carbohydrate) of the three tested cyanobacterial species

GC-MS of the cyanobacterial extracts

The chemical constituents of the three cyanobacterial crude extracts were analyzed using GC-MS, revealing a sum total of 30 various compounds. The identified compounds with their IUPAC name, common name, retention time, molecular formula, and percent peak area were categorized into seven chemical groups (Table 2). The identified chemical groups were esters (14 compounds), hydrocarbons (4 compounds), terpenes (3compounds), ketones (5 compounds), and fatty acids, fatty alcohols, steroids, and others with one compound for each. The highest number of compounds was identified in *A. platensis* (17 compounds), whereas 10 compounds were recorded in *L. boryana* and *L.* sp. (Table 2).

Anticancer activity of the cyanobacterial extracts

The cyanobacterial extracts displayed different cytotoxic activities against Hep-G2 and MCF-7 cell lines. *A. platensis* extract displayed the highest cytotoxic effect against Hep-G2 cells with an IC_{50} value of 14µg.mL^{-1} followed by *L.* sp. with an IC_{50} value of 15µg.mL^{-1} (Fig. 6). On the contrary, in terms of inhibition percentage, the extracts displayed different inhibition percentages with *L.* sp. extract exhibiting the highest percent (73.2%), followed by the extract of *A. platensis* (68%) with a concentration of 100µg.mL^{-1} (Fig. 7). Concerning the MCF-7 cell line (Fig. 8), the extract of *A. platensis* showed the highest cytotoxic effect (IC_{50}= 12.1µg.mL^{-1}) followed by *L.* sp (IC_{50}= 13.2µg.mL^{-1}). *Arthrospira platensis* extract showed inhibition efficiency of up to 91.2% against the MCF-7 cells with a concentration of 100µg.mL^{-1} (Fig. 9). The cited results showed a significant gap in the inhibition efficacy between the extract of *L. boryana* and that of *A. platensis* and *L.* sp. extracts, whereas the extract of *L. boryana* revealed a weak and neglectable inhibition, which did not exceed 20%, against Hep-G2 and MCF-7 cell lines.

Discussion

The cancer treatment strategies depend on the surgical removal of cancerous tissues followed by chemotherapy and/or radiotherapy. No potent medicine is available for cancer treatments, and the currently available drugs cause numerous side effects (Teleanu et al., 2020). As a result, scientific attention focuses on natural products as a new trend for treating cancer. Natural products from medicinal plants and microorganisms, such as actinobacteria and fungi, have been approved and used extensively over the decades in various therapeutic areas (Mans et al., 2000). Cyanobacteria, as a promising natural resource, produce a wide array of secondary metabolites that are known for their curative value and used for the treatment of cancer and different microbial infections (Jones et al., 2010). In addition, cyanobacteria can synthesize nanoparticles that play different biological activities, including anticancer activity (El-Sheekh et al., 2021). Optimization of media and culture condition to obtain maximum yield in the tested species is a great demand of this investigation. The growth of cyanobacteria, in general, depends upon the availability of nitrogen and/or phosphate unless a large number of macro-nutrients and micro-nutrients are available for cyanobacterial growth (Larned 1998; Sun et al., 2018; Abd El-Monem et aL., 2021). The cited results clarify that *L.* sp. *and L. boryana* displayed a high DW and Chl. a content in the modified-No. 2 medium, but the highest DW and Chl. a yield of both organisms were obtained on the 21st day in the standard BG11 medium. This result confirmed that standard BG11 medium is the most suitable medium for most cyanobacterial species given that the growth of cyanobacteria requires an appropriate K^+:Na^+ ratio in the cytoplasm; only standard BG11 medium contained a moderate concentration of Na^+, whereas the other media had high Na^+ content (Nehul, 2014). *Spirulina* medium was the sole medium suitable for *A. platensis*, and the highest biomass and Chl. a yields were observed in this medium on the 21st day. These findings agreed with those of Abd El-Baky & El-Baroty (2012) and are supported by the increased biomass and photosynthetic pigment yield of *A. platensis* (18%) using alkaline medium

(pH 9.0) (Ghasemi et al. 2017; Abdel-Aal & Mofeed, 2020). The carbohydrates, proteins, and lipids derived from cyanobacteria represent a source of broad biomolecules with high potency in therapeutics and biotechnology applications. Hence, the present investigation paid great attention to the biochemical characterization of the tested species. In general, the tested species recorded a high protein content that fluctuated between 40% to 56%, in addition to a moderate carbohydrate content ranging between 22% and 24% of the DW. Finally, a low lipid content was observed between 4% to 6% of the DW, with *A. platensis* showing the highest content of the tested biochemical parameter; these results greatly accord with those of Bensehaila et al. (2015) and Aissaoui et al. (2017). The GC-MS analysis of the three tested cyanobacterial extracts showed the presence of chemical substances with previously known biological activities, including anticancer activity. Among the bioactive chemical compound detected in the tested extracts, the phenolic compound 1,4-benzenediol, 2,6-bis(1,1-dimethylethyl) is characterized by its anticancer activity (Selassie et al., 2005). Phytol (3,7,11,15-tetramethyl-2-hexadecen-1-ol) is a diterpene with different biological activities, including antimicrobial and anticancer activities, and is a precursor of vitamins E and K (McGinty et al., 2010). Pentadecane is a hydrocarbon with anticancer and antimicrobial activities (Bruno et al., 2015); isocaproic acid (4-methyl-pentanoic acid) is a fatty acid with a known antitumor activity (Colombo et al., 2005) in addition to squalene, which is a well-known terpene that has antiviral and anticancer activity and used as a drug for the hepatitis C virus (Vera et al., 2009; Kotelevets et al., 2017; El-Sheekh & Abomohra, 2020).

The organic extracts of *A. platensis* and *L.* sp. reduced Hep-G2 cell proliferation and had a cytotoxic effect at IC_{50} of 14 and 15µg mL^{-1}, respectively. This result agreed with that of Ahmed et al. (2017), who reported the cytotoxic effect of *Plectonema terebrans* and *Cyanothece* sp. against the Hep-G2 cell line. Compared with the control, the undiluted concentration (100µg mL^{-1}) extracts of *L.* sp. and *A. platensis* caused growth inhibition percentages of 73.2% and 69% against the Hep-G2 cell line. This result revealed that the crude extracts of isolated cyanobacteria decreased the cell viability of Hep-G2, consistent with the findings obtained by Akbarizare et al. (2020), who proved the effectiveness of *Spirulina platensis* in inhibiting Hep-G2 cells and as a potential natural anticancer drug against HCC. Agreeably, the extracts of *A. platensis* and *L.* sp. had a more cytotoxic effect against the MCF-7 cell line with IC_{50} values of 12.1 and 13.2µg mL^{-1}, respectively. The extracts of *A. platensis* and *L.* sp. also showed high inhibition percentages of 91.2% and 71%, respectively, against MCF-7 cells at undiluted concentrations. The obtained results agreed with those of Felczykowska et al. (2015), who proved the cytotoxic effect of several cyanobacterial isolates against MCF-7 cells, and those of Karan & Aydin (2018), who proved the effectiveness of *Nostoc linckia* and *Geitlerinem nacarotinosum* in preventing the growth of specific cancer cell lines including MCF-7. The crude extracts of cyanobacteria used in this work exhibited a high potent activity against breast adenocarcinoma and HCC cell lines compared with extensively used chemotherapeutic drugs, such as vinblastine, docetaxel, and tamoxifen with IC_{50} values ranging between 10µm to 50µm, against different types of cancer cell lines (Samadi et al., 2014).

Conflicts of interest: No conflicts of interest have been declared.

Authors' contribution: The authors confirm their contribution to the paper as follows, Mohamed Deyab and Jelan Mofeed design the study. Preparation of draft manuscript performed by Jelan Mofeed and Emad El-Bilawy. Emad El-Bilawy carried out the practical work. All authors reviewed the results and approved the final version of the manuscript.

Ethical approval: Not applicable

Conclusion

The obtained results from this investigation along with those of previous studies reveal the importance of cyanobacteria in manufacturing new pharmaceuticals, especially anticancer drugs. The present study concludes that the methanolic extracts of the Egyptian isolate *A. platensis* and *L.* sp. showed a high *in vitro* anticancer activity of up to 91% inhibition of MCF-7 cells and 73.2% inhibition of the Hep-G2 cell line. The promising results obtained in the present investigation indicate that these isolates represent a potential source of anticancer drugs for the future. However, more in vivo studies are required for their application as an anticancer drug.

TABLE 2. Bioactive compounds present in methanolic extracts of the three tested cyanobacteria

	Chemical Formula	Retention Time (Min)	peak Area%	Chemical group
***Leptolyngbya* sp**				
1,4-Benzenediol, 2,6-bis(1,1-dimethylethyl)-	C14H22O2	18.2	5.83	Keton
Benzoic acid, methyl ester	C8H8O2	21.1	3.93	Ester
4-Hydroxymandelic acid, ethyl ester, di-TMS	C16H28O4Si2	30.9	23.25	Ester
Eicosane	C20H42	32.5	6.92	Hydrocarbon
Nonadecane	C19H40	34	11.6	Hydrocarbon
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	34.7	8.78	Terpen
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C16H22O4	35.1	5.80	Ester
Pentadecanoic acid, 14-methyl-, methyl ester	C16H22O4	35.6	4.01	Ester
1,2-Benzenedicarboxylic acid, butyl octyl ester	C20H30O4	36.4	9.3	Ester
1,2-Benzenedicarboxylic acid, diisooctyl	C24H38O4	45.6	10.46	Ester
Leptolyngbya boryana				
Pentanoic acid, 4-methyl-	C6H12O2	26	15.08	Fatty acid
Acetophenone, 2-chloro-	C8H7ClO	32	3.93	Keton
4-Hydroxymandelic acid, ethyl ester, di-TMS	C16H28O4Si2	30.9	18.43	Ester
Pentadecane	C15H32	33.8	16.2	Hydrocarbon
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	34.7	4.46	Terpen
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C16H22O4	35.1	5.02	Ester
7-Hexadecenoic acid, methyl ester, (Z)-	C17H32O2	35.9	5.58	Ester
Hexadecanoic acid, methyl ester	C17H34O2	37	5.02	Ester
11-Octadecenoic acid, methyl ester	C19H36O2	38.8	7.80	Ester
3',8,8'-Trimethoxy-3-piperidyl-2,2'-binaphthalene-1,1',4,4'-tetrone	C28H25NO7	46.7	11.17	Keton
Arthrospira platensis				
Propiophenone, 2'-(trimethylsiloxy)-	C12O18O2Si	21	1.72	Keton
Benzoic acid, hydrazide	C7H8N2O	23.5	0.86	Other
Octadecanoic acid, 1-[(tetradecyloxy)carbonyl] pentadecyl ester	C19H36O2	25.1	25.1	Ester
3-Methyl-2-(3-methylpentyl)-3-buten-1-ol	C5H10O	25.1	1.72	Fatty Alcohol
Eicosane	C20H42	32.5	1.29	Hydrocarbon
Pentadecane, 7-methyl-	C16H34	30.8	18.96	Hydrocarbon
3,7,11,15-Tetramethyl-2-hexadecen-1-ol	C20H40O	34.7	10.34	Terpenes
1,2-Benzenedicarboxylic acid, bis(2-methylpropyl) ester	C16H22O4	35.1	10.77	Esters
7,9-Di-tert-butyl-1-oxaspiro(4,5)deca-6,9-diene-2,8-dione	C17H24O3	33.4	4.3	Keton
Pentadecanoic acid, 14-methyl-, methyl ester	C17H34O2	35.3	6.46	Ester
Ethaneperoxoic acid, 1-cyano-1-[2-(2-phenyl-1,3-dioxolan-2-yl)ethyl]pentyl ester	C19H25NO5	35.7	7.32	Ester
9,12-Octadecadienoyl chloride, (Z,Z)-	$C_{18}H_{31}ClO$	27.5	1.72	ester
9,12,15-Octadecatrienoic acid, methyl ester, (Z,Z,Z)-	$C_{19}H_{32}O_2$	35.7	7.32	Ester
Phytol	C20H40O	38.6	3.44	Terpene
1,2-Benzenedicarboxylic acid, diisooctyl ester	C24H38O4	45.6	2.15	Ester
Squalene	C30H50	38.8	6.46	Terpene
Cholesterol	C30H50	51.8	4.64	Steroid

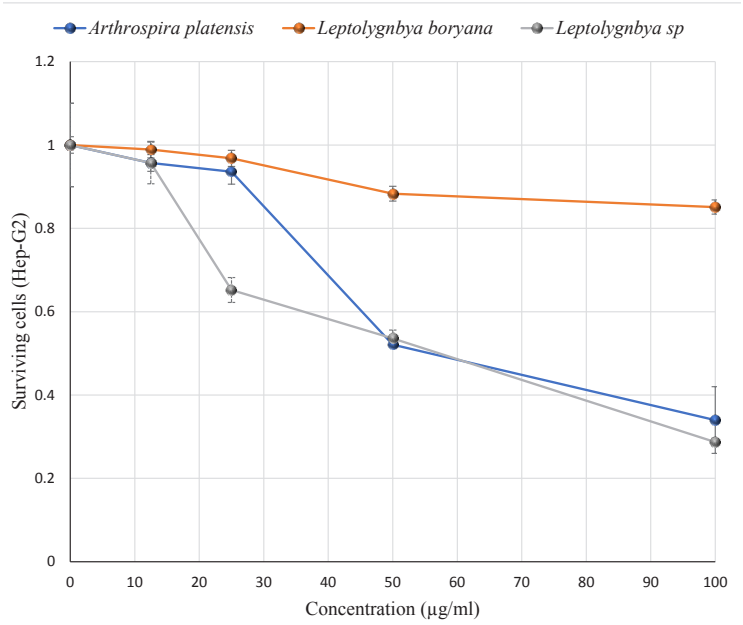

Fig. 6. Dose response curve of the three cyanobacterial extracts against Hep- G2 cell line

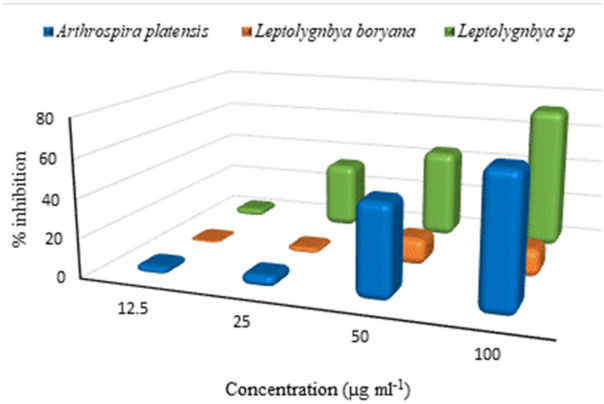

Fig. 7. Inhibitory potential of the tested extract against Hep-G2 cells

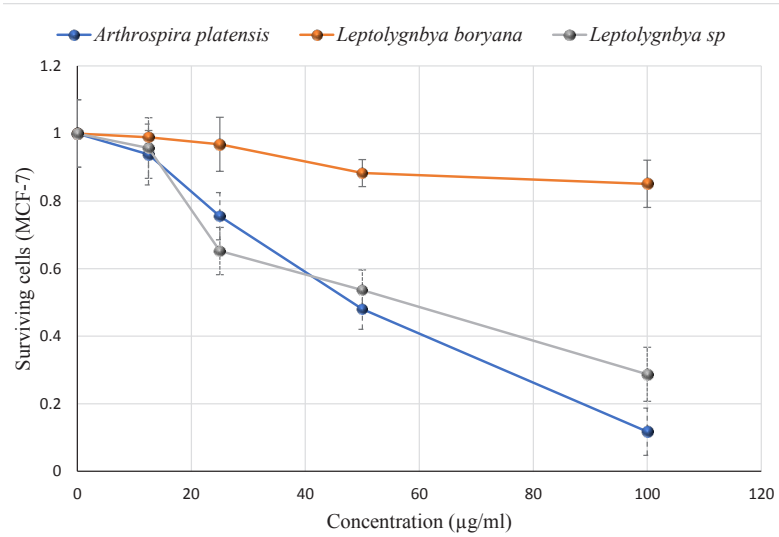

Fig. 8. Dose response curve of the three cyanobacterial extracts against MCF-7 cell line

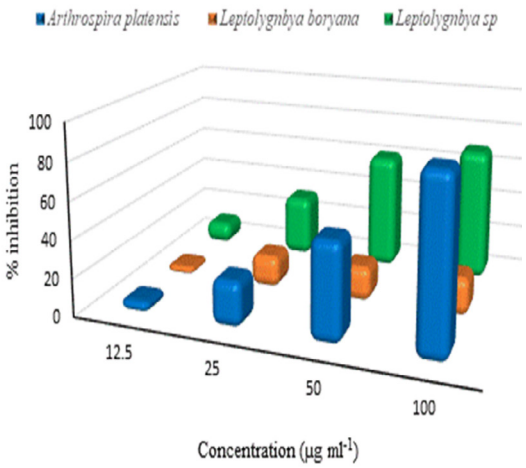

Fig. 9. Inhibitory potential of the tested extract against MCF-7 cells

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اختبار الأثر المضاد للسرطان لثلاث عزلات مصرية من السيانوبكتريا على خلايا سرطان الثدي والكبد معملياً

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تمثل السيانوبكتريا مصدرًا واعدًا لم يتم استكشافه بالكامل لمجموعة واسعة من نواتج الأيض الثانوي والتي تمثل مصدرًا لاكتشاف المركبات والأدوية. في الدراسة الحالية، تم اختبار مستخلصات ثلاثة عزلات مصرية من السيانوبكتريا، وهي *Arthrospira platensis* MH285264 و*Leptolyngbya boryana* MH155317 و *Leptolyngbya* sp. MH285263 ضد خطوط خلايا سرطان الثدي (MCF-7) والخلايا السرطانية الكبدية (Hep-G2). من أجل الحصول على أعلى كتل حيوية من البكتريا المختبرة، تم دراسة نموها باستخدام 8 أوساط غذائية مختلفة .ثم قمنا بتحليل المكون الكيميائي لأعلى كتل حيوية لكل كائن بإستخدام جهاز الفصل الغازي (GC-MS). أثبتت التحاليل الكيميائية وجود عدد من المركبات (نتائج الأيض الثانوي) والتي تعرف بنشاطها المضاد للسرطان. في هذه الدراسة تم اختبار الأثر المضاد للسرطان لمستخلصات العزلات المختلفة معمليا ضد خلايا السرطان الكبدية (Hep-G2) و خلايا سرطان الثدي (MCF-7) بإستخدام طريقة SRB والتي تعبر عن الجزء المتبقي من الخلايا المعالجة بالمستخلصات مقارنة بالخلايا غير المعالجة وكان يقاس النشاط المضاد للخلايا السرطانية بعد 48 ساعة. سجلت مستخلصات العزلتين *A. platensis* و *L.* sp أعلى نشاط مضاد لخلايا السرطان الكبدية (Hep-G2) بتركيز نصفي مثبط IC_{50} (وهو التركيز الذي يقتل 50% من الخلايا المعالجة بالمستخلصات مقارنة بالخلايا غير المعالجة) 14 و 15 ميكروجرام/ مليلتر على الترتيب. عند استخدام أعلى تركيز مستخدم في هذا الأختبار وهو 100 ميكروجرام/مليلتر تم تسجيل نسب تثبيط مختلفة لخلايا (Hep-G2) وكانت أعلى نسبة تثبيط 73,2% لمستخلص عزلة *L.* sp. مستخلصات عزلتي *A. platensis* و *L.* sp سجلتا أعلى نشاط مضاد لخلايا سرطان الثدي (MCF-7) بنسب تثبيط (IC_{50}) 12,1و13,2 ميكروجرام/مليلتر على الترتيب. وعند استخدام أعلى تركيز (100 ميكروجرام/مليلتر) كان أعلى تثبيط لخلايا سرطان الثدي 91.2% بواسطة مستخلص عزلة *A. platensis*. تبشر النتائج الواعدة في هذه الدراسة بوجود بدائل طبيعية ذات كفاءة عالية لإدراجها في استراتيجيات علاج السرطان.