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Cytotoxicity and antibacterial activity of the blue green alga *Microcystis aeruginosa* extracts against human cancer cell lines and foodborne bacteria

Gomaa N. Abdel-Rahman*[a], Yousef Y. Sultan[a], Mohamed A. Hassoub[b] and Diaa A. Marrez[a]

[a] *Food Toxicology and Contaminants Department, National Research Centre, Dokki, Cairo, Egypt*

[b] *Fertilization Technology Department, National Research Centre, Dokki, Cairo, Egypt*

Abstract

Foodborne pathogens are one of the important biological hazards causing food spoilage. Nowadays, both research and industry are looking for natural sources of food preservatives and nutraceutical agents as an alternative of the chemical ones. Algae are one of these resources and they exist abundantly in the Egyptian aquatic environment. The algal biomass of *Microcystis aeruginosa* was used in the present study. Different solvents (deionized water, methanol, ethanol, acetone, chloroform, diethyl ether, ethyl acetate and hexane) were used for extraction the bioactive metabolites from *M. aeruginosa*. Diethyl ether extract (DEE) showed marked antibacterial activity against all tested foodborne pathogenic bacteria achieving inhibition ranged between 13.3 mm with *K. pneumoniae* and 29.3 mm with *P. aeruginosa*. The DEE extract of *M. aeruginosa* was fractionated using column chromatography technique. Among all fractions of diethyl ether extract, F7 had an effect against all tested bacteria. Eight compounds (9,12-Octadecadienoic acid methyl ester, Hexadecadienoic acid methyl ester, 9-Octadecadienoic acid, Nonadecane, Hexadecane, 9,12,15-Octadecadienoic acid, Octasiloxane and 10-Octadecenoic acid, methyl ester) were identified using GC/MS in such fraction. In addition to the antibacterial activity of DEE and F7, they worked as anticancer agents. Low concentration of DEE (<100 μg ml^{-1}) was required at IC_{50} for liver, colon and breast cancer cell lines. Fraction F7 had obvious effect against colon cancer cell line with low IC_{50}, 28.73 μg ml^{-1}. Generally, *M. aeruginosa* had valuable bioactive metabolites which can be used as natural preservatives and anticancer agents.

Keywords: *Microcystis aeruginosa*, cytotoxicity, foodborne bacteria, GC/MS.

Introduction

Food-borne pathogenic bacteria is one of the most common reasons that cause illness and death especially in the developing countries [1]. The most reported foodborne pathogens are associated with Gram negative bacteria including *Pseudomonas aeruginosa*, *Salmonella typhi* and *Escherichia coli*, as well as Gram positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus* [2, 3]. Although, the chemical preservative can achieve desirable prevention of foodborne pathogens, it have negative impacts to human health due to the presence of some residues of these chemicals in food which increase microorganisms resistance against these chemicals [4]. The natural sources of food preservations are one of the most promising applications to prevent the growth of food spoilage microorganisms and increase the shelf life [5].

Cyanobacteria are photoautotrophic microorganisms, which can be cultivated in different natural and artificial media to produce several ingredients such as proteins, pigments, fatty acids, polysaccharides, minerals and vitamins [6, 7]. Also, some species of cyanobacteria are used on large scale production for human nutrition, animal feed, bioremediation, biofuel and pharmaceutical applications [8-10]. In addition to dried cyanobacterial biomass used as food additive, its extracts can be used in food applications as a bio-preservative and nutraceutical agents [11]. A large number of cyanobacterial extracts and/or extracellular products have been found to have antimicrobial activity [12]. The most identified

*Corresponding author e-mail: gomaa.nrc@gmail.com.; (Gomaa N. Abdel-Rahman).

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antibacterial and antifungal substances from cyanobacteria are arealkaloids, phenolics, fatty acids, lipopeptides, terpenoids and cyclic peptides [13, 14]. Also, cyanobacteria have been found to be excellent sources of cytotoxic secondary metabolites as drug discovery anticancer agents [15]. Calcium-Spirulan of *Spirulina* was reported to prevent pulmonary metastasis, as well as the adhesion and proliferation of tumor cells [16]. Borophycin from *Nostoc linckia* and *Nostoc spongiaeforme* exhibits promising antitumor activity against standard cancer cell lines, human epidermoid carcinoma and human colorectal adenocarcinoma cell lines [17]. Also, fatty acids and fatty acids methyl ester from *Oscillatoria brevis* had cytotoxicity against HepG2, MCF7 and HCT116 cancer cell lines [11].

M. aeruginosa is one of the dominant algal species in Egyptian aquatic environment and commonly exists in algal bloom form [18].This study focused on evaluating the antibacterial and anticancer activity of *M. aeruginosa* extracts and its fractions. Identifying the chemical profile of the most effective fraction against foodborne pathogenic bacteria and human cancer cell lines using GC/MS was an advanced aim of this study.

Materials and Methods

1. Cultivation of M. aeruginosa

Pure isolate of *M. aeruginosa* was obtained from Marine Toxins Lab., National Research Centre, Egypt [18] and cultivated on BG-11 medium [19]. The biomass of *M. aeruginosa* (6 x 107 cell ml^{-1}) was harvested (at the stationary phase of growth, 25 days), then dried at 50 ºC overnight in a hot air oven.

2. Preparation of M. aeruginosa extracts

Eight samples of the dried *M. aeruginosa* biomass (20 g each) were homogenized separately in deionized water and different organic solvents (HPLC grade, Sigma-Aldrich) such as chloroform, acetone, diethyl ether, ethanol, methanol, hexane and ethyl acetate. Each homogenized biomass was sonicated using ultrasonic micro tip probe of 400 watt (ULTRASONIC Get 750) for 20 min, then centrifuged for 10 min at 4500 rpm (SIGMA Laborzentrifugen Gmbh). Supernatants of different solvents were collected separately and the remained pellets were re-extracted twice as described before. The supernatants obtained from each solvent were combined, then evaporated at 40º C to dryness using rotary evaporator. Dried extracts were kept in labeled sterile vials at -20 ºC in a deep freezer till the next use [20].

3. Chromatographic fractionations of M. aeruginosa DEE crude extract

The diethyl ether extract (DEE) of *M. aeruginosa* was fractionated using column chromatography technique. Glass column (30 x 500 mm) was initially packed with 5 g of anhydrous sodium sulphate followed by 30 g of silica gel (0.06 - 0.2 mm, 70 - 230 mesh ASTM) using chloroform as a carrier solvent to create slurry. Finally, 5 g of anhydrous sodium sulphate was added to the top of silica gel to prevent column from drying. A portion of DEE (500 mg) in 10 ml chloroform was loaded to the column and allowed to flow at a rate of a drop sec^{-1}. The silica gel column was eluted with different mixture (v/v) of chloroform: methanol (98:2), (95:5), (90:10), (80:20), (50:50), (25:75) and finally methanol 100% to give 7 fractions. The fractions, 50 ml each, were collected, evaporated under vacuum and stored for further analysis and bioassays [11].

4. Antibacterial activity

4.1. Test microorganisms

Foodborne pathogenic bacteria such as *Escherichia coli* 0157 H7 (ATCC 51659), *Salmonella typhi* (ATCC 25566), *Klebsiella pneumoniae* (LMD 7726) and *Pseudomonas aeruginosa* (NRRL B-272) as Gram negative bacteria, as well as *Staphylococcus aureus* (ATCC 13565) and *Bacillus cereus* (EMCC 1080) as Gram-positive bacteria were used for evaluation the antibacterial activity of *M. aeruginosa* crude extracts and its fractions.

4.2. Disc diffusion method

Each bacterial species was incubated at nutrient agar slant for 24 h, then inoculated (a loop full) in a tube containing 5 ml of tryptic soy broth and incubated for 2-6 h at 35º C until obtain the suitable turbidity (0.5 McFarland BaSO4 Standard). Disc diffusion method of Kirby-Bauer technique [21] was used for examination the antibacterial activity of *M. aeruginosa* crude extracts and its diethyl ether fractions against all the tested bacterial species. Using cotton swabs, nutrient agar plates were uniformly inoculated with tryptic soy broth of bacterial cultures. Ten mg of each extract and fraction were dissolved in 1 ml of dimethyl sulfoxide (DMSO) to obtain 10 mg ml^{-1} concentration. Diethyl either extracts or fractions were loaded on Sterilized discs (6 mm) from Whatman No. 1 filter paper, then dried completely under sterile conditions. The discs were placed on the seeded plates by using a sterile forceps. Tetracycline (500 mg ml^{-1}) and DMSO represented the positive control and negative control, respectively. Inoculated plates were incubated for 24 h at 37 ºC, then measure

the inhibition zones that expressed as the diameter of clear zone including the diameter of the paper disc.

4.3. Examination of minimum inhibitory concentration (MIC)

The MIC examination was achieved according to tube dilution method [22]. The tested bacterial species (24 h culture) was diluted in tryptic soy broth (10 ml) with reference to the 0.5 McFarland standard to obtain inocula of 108 cfu ml^{-1}. Nine different concentrations (4.0, 3.0, 2.0, 1.5, 1.0, 0.75, 0.50, 0.25, 0.1 mg ml^{-1} in DMSO) of each *M. aeruginosa* ether extract and fraction F7 were prepared in culture tubes. Each tube was inoculated with 100 µl of bacterial cell suspension, then incubated for 24 h at 37 ºC. The inoculum growth of tested bacteria was observed by turbidity of the broth and the minimum inhibitory concentration (MIC) was taken according to the lowest concentration of the extract which inhibited the growth of the tested microorganism.

5. In vitro cytotoxicity assay

The colorimetric method of Mosmann [23] was used as in vitro cytotoxicity assay at the Bioassay-Cell Culture Laboratory, National Research Centre. *M. aeruginosa* diethyl ether extract and fraction F7 were evaluated as anticancer against three human cancer cell lines i.e. colon cancer (HCT116), hepatocellular carcinoma (HepG2) and breast cancer (MCF7). Cells were suspended in RPMI 1640 medium in 96-well microtiter plastic plates at concentration of 10 x 10^3 cells/well and kept for 24 h at 37 ºC under 5% CO2 using a water jacketed carbon dioxide incubator (Sheldon, TC2323, Cornelius, OR, USA).

Media was aspirated, fresh medium (without serum) was added and cells were incubated for 48 h, either alone (negative control) or with different concentrations of either extract or fraction (0.78, 1.56, 3.125, 6.25, 12.5, 25, 50, 100, 150 and 200 µg ml^{-1}). The medium was aspirated 40 µl MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) of 2.5 µg ml^{-1} was added to each well and incubated for further four hours at 37 ºC under 5% CO_2. To stop the reaction and dissolving the formed crystals, 200 µl of 10% Sodium dodecyl sulphate (SDS) in deionized water was added to each well and incubated overnight at 37 ºC. A positive control composed of Novantron standard (100 µg ml^{-1}) was used as a known cytotoxic natural agent that gives 100% lethality under the same conditions [24].

The absorbance was then measured using a microplate multi-well reader (Bio-Rad Laboratories Inc., model 3350, Hercules, California, USA) at 595 nm and a reference wavelength of 620 nm. A statistical significance was tested between samples and negative control (cells with vehicle) using independent t-test by SPSS 11 program. DMSO is the vehicle used for dissolution of plant extracts and its final concentration on the cells was less than 0.2%. The percentage of change in viability was calculated according to the formula, ((Reading of extract/Reading of negative control) -1) x 100. A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

6. GC/MS analysis

The diethyl ether fraction F7 were subjected to analysis of chemical composition by using GC/MS, Thermo Scientific, Trace GC Ultra coupled with ISQ Single Quadrupole mass spectrometer (MS). Components were separated by using TG-5MS fused silica capillary column (30 m, 0.251 mm, 0.1 mm film thickness). Helium was used as carrier gas at a constant flow rate of 1 ml min^{-1}. The injector and MS transfer line temperature was set at 280 ºC. The oven temperature program was started at 50 ºC for 2 min. Then the temperature was ramped to 150 ºC at 7 ºC min^{-1}, then to 270 ºC at 5 ºC min^{-1} and held for 2 min, finally to 310 ºC at 3.5 ºC min^{-1} and held for 10 min. Mass Spectra were recorded under ionization energy of 70 eV [11]. A tentative identification of the compounds was performed based on the comparison of their relative retention time and mass spectra with those of the NIST, WILLY library data of the GC/MS system. The quantification of all identified components was investigated using a percent relative peak area.

7. Statistical analysis

Statistical significance was determined using Statistica Version 9 (State Soft, Tulsa, Okla., USA). The means were determined by analysis of variance (ANOVA, one way analysis) ($p < 0.05$). Followed by Fisher's LSD (Least significant differences) method ($\alpha = 0.05$) to compare significant differences between treatments.

Results and discussion

1. Antibacterial activity of M. aeruginosa crude extracts

Table (1) illustrates *M. aeruginosa* crude extracts antibacterial activity against different species of foodborne pathogenic bacteria. *M. aeruginosa* methanolic, diethyl ether, chloroform and ethyl acetate extracts showed antibacterial activity against the tested bacteria, followed by ethanolic extract which had antibacterial activity against all tested bacteria except *K. pneumoniae*. While, hexane extract

had antibacterial activity only against *S. typhi*. In contrast, *M. aeruginosa* aqueous extract have no activity against all tested bacteria. Diethyl ether extract showed the highest inhibition zone as 29.3 mm against *P. aeruginosa* followed by 26.0 mm against *E. coli*.

M. aeruginosa MDEG1 strain petroleum ether and chloroform extracts showed antibacterial activity against Gram positive bacteria *S. aureus*, *B. cereus* and Gram negative bacteria *E. coli*, *P. aeruginosa* and *K. pneumoniae* [25]. Also, Padhi et al. [26] reported that both ether and aqueous extracts of *M. aeruginosa* had no activity against *B. cereus*, *E. coli*, *Klebsiella* sp. and *Pseudomonas* sp. Silva-Stenico et al. [27] indicated that *M. aeruginosa* NPCD-1 ethyl acetate, methanolic aliquots and its non-microcystin-producer withdraw showed a strong antibacterial activity against *B. subtilis*, *B. cereus*, *S. typhimurium*, *S. aureus* and *E. coli*. Al-Wathnani et al. [28] found that *M. aeruginosa* acetone extract had antibacterial activity against *Staphylococcus aureus* and *Bacillus subtilis*. The present study reported that *M. aeruginosa* diethyl ether extract had the highest antibacterial activity against all tested bacteria; this finding is in agreement with the study conducted by Marrez and Sultan [29] which found that *M. aeruginosa* diethyl ether extract showed the greatest antifungal against different species of mycotoxigenic fungi among all tested extracts.

TABLE 1. Antibacterial activity of *Microcystis aeruginosa* crude extracts.

Bacteria	Inhibition zone mm (Mean±*S.E)									
	-ve Control	+ve Control	Aqueous	MeOH	EtOH	Acetone	CH_3Cl	DEE	EtOA	Hexane
B. cereus	--	18.6±1.1^{b}	--	9.0±1.3^{d}	8.8±0.9^{d}	8.5±0.6^{d}	10.5±0.9^{c}	23.2±1.6^{a}	10.0±1.0^{c}	--
Staph. aureus	--	16.0±1.0^{b}	--	9.2±0.6^{cd}	8.5±0.9^{d}	9.2±0.8^{cd}	9.2± 0.8^{cd}	22.3±2.5^{a}	9.7±1.6^{c}	--
E. coli	--	16.8±1.3^{b}	--	9.3±1.3^{d}	9.2±0.8^{d}	9.5±0.8^{d}	10.2±1.0^{c}	26.0±1.8^{a}	9.2±0.3^{d}	--
P. aeruginosa	--	18.5±1.4^{b}	--	7.8±0.3^{e}	9.3±0.6^{c}	7.8±0.3^{e}	8.0±0.5^{d}	29.3±3.8^{a}	8.7±1.0^{c}	--
S. typhi	--	17.2±0.6^{b}	--	8.8±0.8^{d}	8.5±0.9^{de}	--	12.3±0.6^{c}	22.5±1.3^{a}	8.2±0.3^{e}	7.3±0.3^{f}
K. pneumoniae	--	16.5±0.5^{a}	--	7.5±0.6^{d}	--	--	8.8±0.8^{c}	13.3±0.8^{b}	9.3±0.8^{c}	--

n = 3, *S.E: standard error, different subscripts within row (a, b, c, d and e) are significantly different at the 5% level, –: No inhibition, MeOH: methanol, EtOH: ethanol, DEE: diethyl ether, EtOA: ethyl acetate, negative control: DMSO, positive control: tetracycline.

2. Antibacterial activity of M. aeruginosa diethyl ether fractions

Diethyl ether extract of *M. aeruginosa* was chosen for further investigation due to the highest antibacterial activity among the other extracts. Diethyl ether extract was fractionated to 7 fractions using silica gel column chromatography. *M. aeruginosa* diethyl ether fractions antibacterial activity against foodborne pathogenic bacteria is represented in Table (2). Fraction F7 showed high antibacterial activity against the tested bacteria followed by F4 which had activity against all tested bacteria except, *S. typhi* and *B. cereus*, as well as F6 which showed bioactivity against most tested bacteria except *Staph. aureus* and *K. pneumoniae*. In contrast, fractions F1, F2, F3 and F5 observed no antibacterial activity against all tested bacteria at the tested concentrations. The highest inhibition zone, 11.8 mm, was showed using F7 against *Staph. aureus*, while the lowest activity, 7.0 mm, was observed against *K. pneumoniae* by both F4 and F7.

The present results are in agreement with those of Marrez et al. [11] who found that the fractions of *Oscillatoria brevis* diethyl ether extract showed antibacterial activity against *B. cereus*, *Staph. aureus*, *S. typhi*, *E. coli*, *P. aeruginosa* and *K. pneumoniae*. Pradhan et al. [30] reported that out of 7 *M. aeruginosa* polar fractions 4 fractions were effective against *P. putida*, *P. aeruginosa*, *P. fluorescens*, *E. coli* and *E. tarda*. Marrez et al. [18] indicated that *M. aeruginosa* diethyl ether fractions had antifungal activity against different species of mycotoxigenic fungi.

TABLE 2. Antibacterial activity of *Microcystis aeruginosa* DEE extract fractions.

Bacteria	Inhibition zone mm (Mean±*S.E)								
	-ve Control	+ve Control	F1	F2	F3	F4	F5	F6	F7
B. cereus	--	18.5±1.6[a]	--	--	--	--	--	8.3±1.2[b]	7.7±1.2[c]
Staph. aureus	--	16.2±1.0[a]	--	--	--	7.7±1.2[c]	--	--	11.7±2.1[b]
E. coli	--	16.5±0.9[a]	--	--	--	7.7±0.6[c]	--	9.0±1.5[b]	9.7±1.2[b]
P. aeruginosa	--	18.7±1.3[a]	--	--	--	7.3±0.6[c]	--	7.3±0.6[c]	9.3±0.6[b]
S. typhi	--	18.0±1.0[a]	--	--	--	--	--	9.0±0.7[b]	7.3±0.6[c]
K. pneumoniae	--	16.2±1.1[a]	--	--	--	7.0±0.1[b]	--	--	7.0±0.3[b]

n = 3, *S.E: standard error, different subscripts within row (a, b and c) are significantly different at the 5% level, –: No inhibition, negative control: DMSO, positive control: tetracycline.

3. MIC values of M. aeruginosa DEE extract and fraction F7

Figure 1 illustrates the minimum inhibitory concentration (MIC) of *M. aeruginosa* DEE and fraction F7 against the tested foodborne pathogenic bacteria. The highest activity of *M. aeruginosa* DEE extract was observed against *E. coli* and *S. typhi* with MIC values 0.5 and 0.6 mg ml^{-1}, respectively, while, the lowest MIC value was recorded against *Staph. aureus* and *K. pneumoniae* as 1.2 and 1.1 mg ml^{-1}, respectively. On other hand, the highest activity from fraction F7 was showed against *S. typhi* with MIC value 0.8 mg ml^{-1} and the lowest activity was observed against *Staph. aureus* with 1.7 mg ml^{-1} MIC Value.

Ishida et al. [31] found that *M. aeruginosa* peptide fraction inhibited the growth of Gram-positive bacteria *Staph. aureus* at concentration 1 µg ml^{-1}.The antibacterial activity of various *M. aeruginosa* extracts was studied against some pathogenic bacteria. Methanolic, chloroform and aqueous extracts of *M. aeruginosa* had antibacterial activity against 2 strains of *P. putida*, 2 strains of *P. aeruginosa*, 2 strains of *P. fluorescens* and 6 strains of *E. coli* with MIC values ranged from 0.075 to 44 mg ml^{-1} [30]. Ramos et al. [32] reported that aqueous, hexane, chloroform and methanol extracts of *M. aeruginosa* RST 950 observed antibacterial activity against sensitive and resistant strains of *M. tuberculosis* with MIC values ranged between 1.93 µM and 0.06 µM. On other hand, Marrez et al. [33] indicated that both *Scenedesmus obliquus* diethyl ether extract and its fractions showed antibacterial activity against *B. cereus*, *Staph. aureus*, *P. aeruginosa*, *S. typhi*, *E. coli* and *K. pneumoniae* with MIC values ranged between 0.5 and 1.9 mg ml^{-1}.

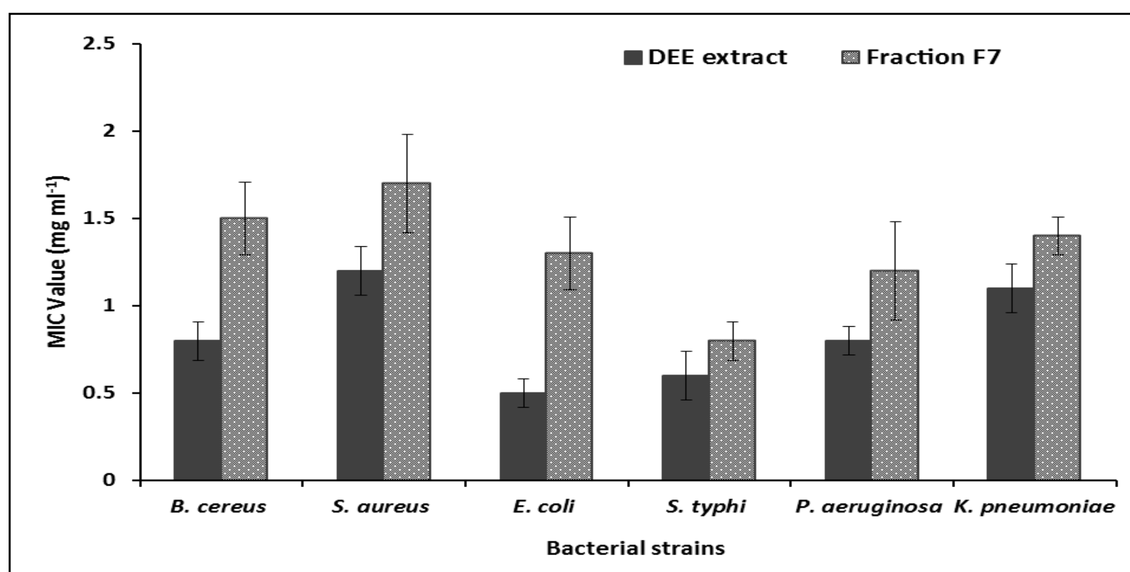

Fig. 1. MIC values (mg ml^{-1}) of *M. aeruginosa* DEE extract and fraction F7.

4. Cytotoxic activity of M. aeruginosa DEE extract and fraction F7

Since, *M. aeruginosa* diethyl ether extract had antibacterial activity against all tested bacteria. This extract was tested for their effectiveness as anticancer agent on liver cancer cell lines HepG2, colon cancer cell lines HCT116 and breast cancer cell lines MCF7 (Fig. 2). The anticancer activity of *M. aeruginosa* DEE extract was obviously clear against the viability of HCT116 and HepG2 cell lines with low IC_{50} values as 42.97 and 63.25 µg ml^{-1}, respectively. Whereas, *M. aeruginosa* DEE extract had lower anticancer activity against MCF7 cell lines with high IC_{50} value as 90.7 µg ml^{-1}. Figure 3 illustrates the cytotoxicity of fraction F7 DEE extract against HepG2, HCT116 and MCF7. Interestingly, its efficiency as anticancer agent against HCT116 cells increased when compared with the crude extract of DEE, where recording low IC_{50} value as 28.72 µg ml^{-1}. However, it had no activity against HepG2 and MCF7 cell lines even at high concentration, 1 mg ml^{-1}.

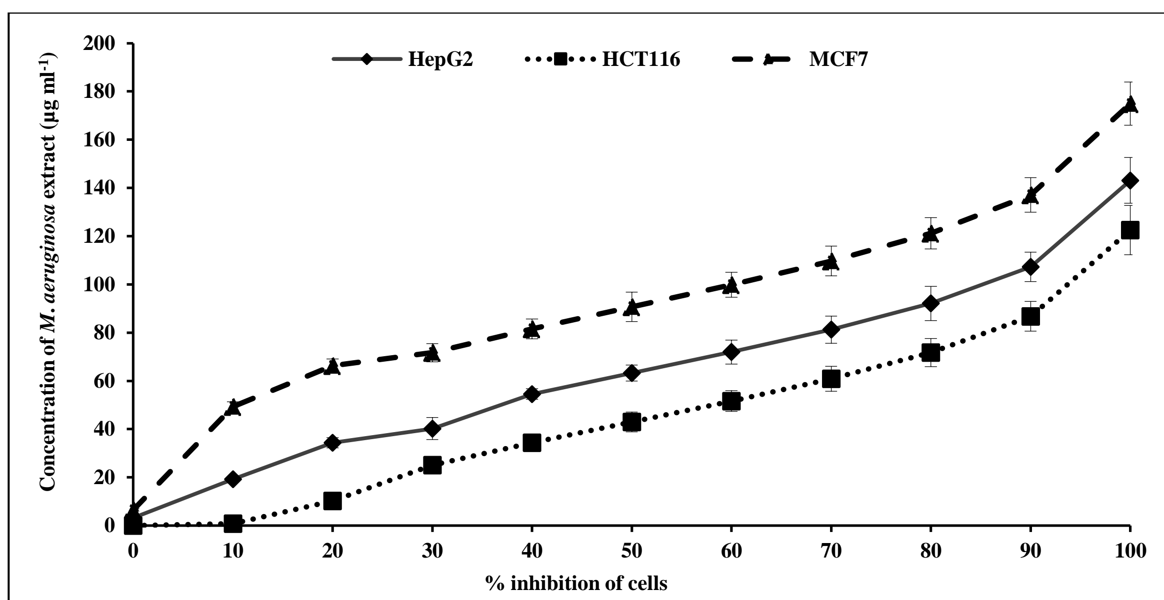

Fig. 2. Cytotoxic assay of *M. aeruginosa* DEE extract on HepG2, HCT116 and MCF7 cell lines.

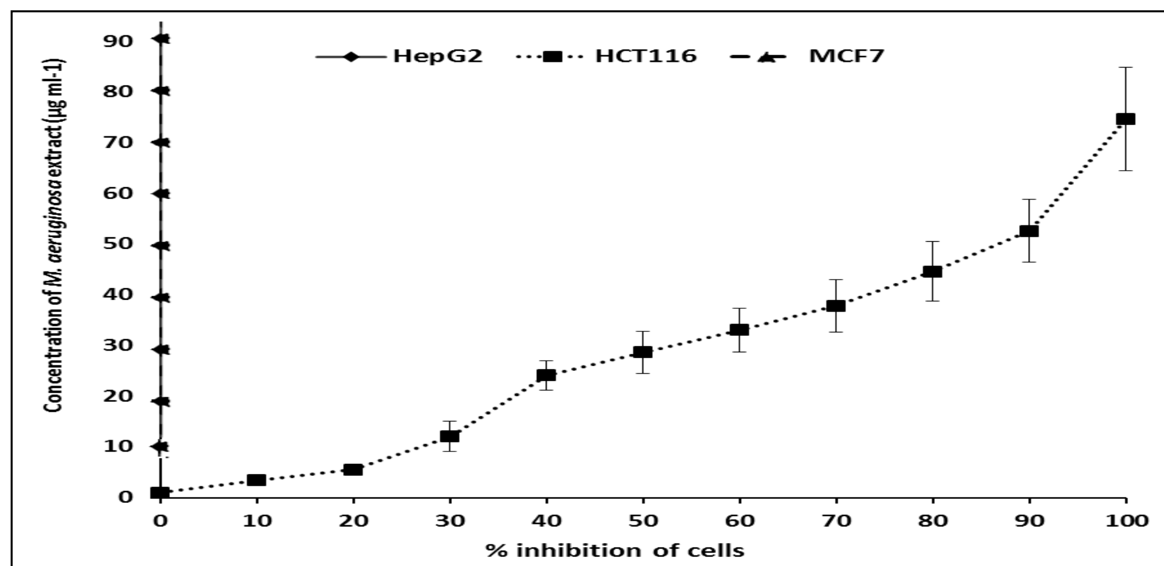

Fig. 3. Cytotoxic assay of *M. aeruginosa* DEE extract fraction F7 on HepG2, HCT116 and MCF7 cell lines.

Silva-Stenico et al. [34] found that *M. aeruginosa* NPCD-1 methanolic extract and *M. aeruginosa* NPJB-1 ethanolic extract had anticancer activity against lung cancer (3LL) and colon carcinoma (CT-26), respectively, whereas *M. aeruginosa* NPLJ-4 dichloromethane showed anticancer activity against both 3LL and CT-26 cell lines. Felczykowska et al. [35] found that ethanolic extract of *M. aeruginosa* obtained from Baltic Sea had anticancer activity toward cancer cells of breast and uterine cervix origin, but not activity was found against normal dermal fibroblasts. Also, Dias et al. [36] reported that *M. aeruginosa* extract had cytotoxic effect against mice (AML12), monkey (Vero) and human (HepG2) cell lines. In contrast, Deyab et al. [37] indicated that *M. aeruginosa* petroleum ether and chloroform fractions had not anticancer activity against HepG2 cell lines. Ramos et al. [32] revealed that no cytoxicity of *M. aeruginosa* RST 950 aqueous, hexane, chloroform and methanol extracts against HCT cell lines.

5. GC-MS analysis of bioactive compounds in fraction F7

Eight compounds from *M. aeruginosa* DEE extract fraction F7 were identified by GC-MS (Table 3). These compounds were 9,12-Octadecadienoic acid methyl ester with peak area percent 4.6%, Hexadecadienoic acid methyl ester 21.82%, 9-Octadecadienoic acid 2.95%, Nonadecane 15.36%, Hexadecane18.62%, 9,12,15-Octadecadienoic acid 3.18%, Octasiloxane 3.07% and10-Octadecenoic acid, methyl ester 29.64%.

TABLE 3. GC-MS analysis of components detected in *M. aeruginosa* DEE extract fraction F7.

No.	RT	Compound	Area %	Biological activity	References
1	23.08	9,12-Octadecadienoic acid, methyl ester	4.6	Antibacterial, antifungal, anticancer, hepatoprotective, anti-inflammatory	Yu et al. [38]
2	25.33	Hexadecadienoic acid, methyl ester	21.82	Antimicrobial, antioxidant, anticancer	Zito et al. [39]
3	25.73	9-Octadecadienoic acid Z-(CAS)	2.95	Antibacterial, antifungal, antioxidant, anticancer	Jain et al. [40]
4	27.12	Nonadecane	15.36	Antimicrobial, anticancer	Marrez et al. [33]
5	27.43	Hexadecane	18.62	Antibacterial, antifungal	Moustafa et al. [41]
6	31.92	9,12,15-Octadecadienoic acid	3.18	Antimicrobial, anticancer, hepatoprotective, anti-inflammatory	Banu and Nagarajan [42]
7	34.66	Octasiloxane	3.07	Antibacterial, antifungal	Zoué et al. [43]
8	35.38	10-Octadecenoic acid, methyl ester	29.64	Antibacterial, antifungal, antioxidant, decrease blood cholesterol	Belakhdar et al. [44]

Marrez et al. [11] found that 9,12-Octadecadienoic acid, methyl ester, 9-Octadecadienoic, methyl ester and Octasiloxane from blue green alga *Oscillatoria brevis* DEE fraction had antimicrobial activity against foodborne pathogens and human cancer cell lines HepG2, HCT116 and MCF7. Also, Marrez et al. [33] indicated that 9-Octadecadienoic acid, Nonadecane, Hexadecane, 9,12,15-Octadecadienoic acid and Octasiloxane from green alga *Scenedesmus obliquus* diethyl ether fraction showed antifungal activity against mycotoxigenic fungi, antibacterial activity against foodborne pathogens and anticancer activity against HepG2, HCT116 and MCF7 cell lines. Kumar et al. [45], Jain et al. [40] and Govindappa et al. [46] reported that 9, 12-Octadecadienoic acid, methyl ester and 9-Octadecenoic acid, methyl ester isolated from some plants and *S. platensis* displayed antimicrobial activity against several human pathogenic microorganisms.

Abou-Elela et al. [47] reported that Hexadecanoic acid methyl ester from the brown alga *Cytosoria compressa* chloroform extract had broad spectrum antimicrobial effect against different pathogenic bacteria. Zito et al. [39], Asghar et al. [48], Hema et al. [49] and Hsouna et al. [50] found that Hexadecanoic acid, Hexadecanoic acid methyl ester, Octadecenoic acid methyl ester and 9-Octadecenoic acid methyl ester from different medicinal plants exhibited antibacterial, antifungal, antioxidant and anticancer activity. Jain et al. [40], Zoue et al. [43] and Moustafa et al. [41] reported that Octasiloxane and 9, 12-Octadecadienoic acid methyl ester isolated from some plants were known to have potential antibacterial and antifungal activity. Devi and Muthu [51] indicated that 9, 12-Octadecadienoic acid methyl

ester from plant *S. spontaneum* had antimicrobial, anti-inflammatory, anticancer, hepatoprotective and antihistaminic effect.

Conclusion

Development of new antibacterial and anticancer classes as food preservatives and nutraceutical ingredients from natural sources is significant important. The cyanobacterium *M. aeruginosa* DEE extract was found to have the most potent antibacterial and anticancer properties. Moreover, fraction F7 can be considered a specific agent targeted colon cancer cells HCT116. Also, eight valuable bioactive compounds were identified from fraction F7 ranged between fatty acids and fatty acid methyl ester. Lastly, the metabolites of *M. aeruginosa* can be used either to increase food shelf life or as health protector.

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السمية الخلوية والنشاط المضاد للبكتريا من مستخلصات الطحالب الخضراء المزرقة *Microcystis aeruginosa* ضد خطوط الخلايا السرطانية فى الإنسان وبكتيريا الفساد الغذائى

جمعه نورالدين عبد الرحمن [1]، يوسف ياسين سلطان [1]، محمد أحمد حسوب [2] و ضياء عطية مريز [1]

1- قسم سموم وملوثات الغذاء - المركزالقومى للبحوث - الدقى - الجيزة – مصر.
2- قسم تكنولوجيا التسميد - المركزالقومى للبحوث - الدقى - الجيزة – مصر.

تمثل بكتريا الفساد الغذائى التى تنتقل عن طريق الأغذية أحد أهم المخاطر البيولوجية التى تسبب فساد الغذاء والأمراض المزمنه للإنسان. وفى الوقت الحالى يتطلع كل من البحث العلمى والصناعة لإستخدام المصادر الطبيعية كمواد حفظ طبيعية للأغذية كبديل للمواد الكيميائية. وتعد الطحالب أحد أهم هذه الموارد وهى موجودة بكثرة في البيئة المائية المصرية. وقد تم إستخدام الكتلة الحيوية الطحلبية *Microcystis aeruginosa* في هذه الدراسة. وتم استخدام مذيبات مختلفة (ماء منزوع الايونات، الايثانول، الميثانول، داى إيثيل إيثر، هكسان، أسيتون، كلوروفورم، إيثيل أسيتات) لإستخلاص المواد الفعالة الحيوية من *M. aeruginosa*. وسجل مستخلص داى إيثيل إيثر أعلى نشاط مضاد بكتيرى ضد جميع أنواع البكتريا المسببة للأمراض والفساد الغذائى المختبرة، حيث حقق مساحة تثبيط تراوحت من 13.3 مم ضد *K. pneumoniae* إلى 29.3 مم ضد *P. aeruginosa*. وتم تجزئة مستخلص داى إيثيل إيثر باستخدام العمود الكروماتوجرافي إلى 7 أجزاء، وكان Fraction 7 له تأثير مثبط ضد جميع البكتريا المختبرة، وقد تم تعريف ثمانية مركبات باستخدام جهاز التحليل الكروماتوجرافى الغازى المزود بطيف الكتلة GC/MS فى هذا الجزء. وهذه المركبات تراوحت ما بين أحماض دهنية والميثيل أستر للأحماض الدهنية. أيضاً تم إستخدام مستخلص داى إيثيل إيثر وكذلك Fraction 7 كعوامل مضادة للسرطان. وكان أقل تركيز من داى إيثيل إيثر (أقل من 100 ميكروجرام/مل) كافياً كجرعه نصف مثبطة (IC_{50}) لخطوط خلايا سرطان الكبد والقولون والثدى. وكان Fraction 7 له تأثير واضح ضد خط خلايا سرطان القولون مع إنخفاض قيمة الجرعه النصف مثبطة إلى 28.73 ميكروجرام/مل. وعموماً يحتوى الـ *M. aeruginosa* على مواد فعالة حيوية ذات قيمه عالية يمكن إستخدامها كمواد حفظ طبيعية وعوامل مضادة للسرطان.