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Research Article

Biological Activity of the Cyanobacterium *Oscillatoria brevis* Extracts as a Source of Nutraceutical and Bio-preservative Agents

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Abstract

Background and Objective: Recently nutraceutical and bio-preservative agents from algae, as a natural source have received a great attention. The study aimed to evaluate the antibacterial, antifungal activities and the cytotoxicity, of both *Oscillatoria brevis* extracts and their fractions. **Materials and Methods:** Different extracts of *O. brevis* were examined for their activities against different types of food pathogens and mycotoxigenic fungi. *In vitro* cytotoxicity assay against human hepatocellular carcinoma cell line (HepG2), colon cancer cell line (HCT116) and breast cancer cell line (MCF7) was monitored. Experimental results were expressed as Mean±Standard Error for three replicates using SAS 6.03. **Results:** Diethyl ether crude extract (DEE) and diethyl ether fraction No. 4 (F4) inhibited the growth of all tested bacterial and fungal species, where the inhibition zone ranged from 10.2 to 32 mm and from 7 to 10 mm, respectively. Regarding to the *in vitro* cytotoxicity, DEE exhibited high activity against HCT116 and MCF7 cell lines, with IC_{50} values of 22.0 and 39.7 μg mL^{-1}, respectively. Interestingly, the cytotoxicity of F4 against MCF7 cell lines was doubled with an IC_{50} value of 20.6 μg mg^{-1}. The GC/MS analysis revealed the presence of 10 compounds in the F4, most of them have been reported as bioactive agent against different pathogenic bacterial and fungal strain. The detected cytotoxicity was attributed to the presence of phenol, 2,4-bis(1,1- dimethylethyl)-, 9,12-octadecadienoic acid, methyl ester and quercetin. **Conclusion:** It is concluded that diethyl ether extract of *O. brevis* had high antimicrobial activity against common food pathogens. It had also anticancer activity especially against breast cancer cell lines.

Key words: *Oscillatoria brevis*, GC/MS, antibacterial, antifungal, cytotoxicity, nutraceutical, bio-preservative

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Food preservatives are group composed of antimicrobials, antioxidants and antibrowning agents. The antimicrobials are added to food for control natural spoilage of food and/or to avoid contamination by microorganisms, including pathogenic bacteria and fungi[1]. Most of the foodborne pathogens affecting food include *Bacillus cereus*, *Brucella* spp., *Campylobacter* spp., *Clostridium botulinum*, *Escherichia coli*, *Listeria monocytogenes*, *Salmonella* spp., *Shigella* spp., *Staphylococcus aureus* and *Yersinia enterocolitica*[2]. Certain types of fungi produce mycotoxins in food as secondary metabolites called mycotoxigenic fungi. The mycotoxins of greatest concern to food and feed safety are produced primary by three genera of filamentous fungi: *Aspergillus*, *Fusarium* and *Penicillium*[3].

One of the most significant applications of natural antimicrobial substances is food bio-preservation, as the food safety concern. The antimicrobial agents from natural sources have been successfully applied as food additives to eliminate pathogens and food spoilage microorganisms for increasing the food shelf life[4,5].

Cyanobacteria are a diverse group of prokaryotic microscopic cells that can grow rapidly due to their simple structure. They are unicellular species which exist individually or in chains or in groups that capable to convert solar energy to chemical energy via photosynthesis. Cyanobacteria are found in a wide range of different habitats from fresh to marine and hyper-saline environments[6].

Cyanobacteria have a significant attraction as natural source of bioactive molecules with a broad range of biological activities including antibacterial, antifungal, antialgal, antiviral, anticancer, antioxidant and anti-inflammatory effects[7,8]. Some Cyanobacteria species used in nutraceutical industries as health foods and nutrition supplements with various health benefits including enhancing immune system activity, antitumor effects and growth promotion, due to their protein, vitamins, active polysaccharides, pigments and other bioactive compounds[9,10]. Besides, some cyanobacteria such as *Spirulina* sp. have been utilized in aquaculture and animal feed to provide excellent nutritional conditions[11,12]. Also, Cyanobacteria are useful for bioremediation of agro-industrial wastewater and as a biological tool for assessment and monitoring of environmental toxicants such as heavy metals, pesticides and pharmaceuticals[13-15].

Despite the fact that *Oscillatoria brevis* is the predominant species in the most algal bloom formed in Egypt[16], none of the previous work examined either its antimicrobial or anticancer activities. So, the present study aimed to evaluate the antibacterial, antifungal and anticancer activities of both *Oscillatoria brevis* extracts and its fractions. Furthermore, it aimed to identify the chemical profile of the most effective fraction against various microbes and human cancer cell lines by using GC/MS technique.

MATERIALS AND METHODS

Cyanobacterial strain and culture medium: Pure isolate of *Oscillatoria brevis* cyanobacteria was obtained from Marine Toxins Laboratory, National Research Centre, Egypt[17]. The culture medium used for cultivation was BG-11[18]. At the stationary phase of growth, 25 days, *O. brevis* biomass was harvested and dried overnight in a hot air oven at 50°C.

Preparation of *O. brevis* crude extracts: The dried *O. brevis* biomass (20 g) was homogenized separately in water and different organic solvents such as methanol, ethanol, acetone, chloroform, diethyl ether, ethyl acetate and hexane (Analytical grade, Fisher, loughborough, UK). Each homogenized biomass was sonicated for 20 min using ultrasonic micro-tip probe of 400 watt, then centrifuged at 4500 rpm for 10 min. Supernatants were collected separately and the pellets were re-extracted twice as mentioned before. Combined supernatants were evaporated to dryness at 40°C using rotary evaporator. Dried extracts were kept in labeled sterile vials at -20°C till further use[19].

Antimicrobial assay

Test microorganisms: The antimicrobial activity of *O. brevis* crude extracts were assayed against six species of pathogenic bacteria, two Gram-positive bacteria *Bacillus cereus* EMCC 1080 and *Staphylococcus aureus* ATCC 13565 and four Gram-negative bacteria *Salmonella typhi* ATCC 25566, *Escherichia coli* 0157 H7 ATCC 51659, *Pseudomonas aeruginosa* NRRL B-272 and *Klebsiella pneumoniae* LMD 7726. Nine fungal species were used for antifungal assay, *Aspergillus flavus* NRRL 3357, *A. parasiticus* SSWT 2999, *A. westerdijikia* CCT 6795, *A. steynii* IBT LKN 23096, *A. ochraceus* ITAL 14, *A. carbonarius* ITAL 204, *Fusarium verticillioides* ITEM 10027, *F. proliferatum* MPVP 328 and *Penicillium verrucosum* BFE 500.

Disc diffusion method

Antibacterial assay: From the 24 h incubated nutrient agar slant of each bacterial species a full loop of the microorganism was inoculated in a tube containing 5 mL of tryptic soy broth. The broth culture was incubated at 35°C for 2-6 h until it

achieved the turbidity of 0.5 McFarland $BaSO_4$ standard. The bioactivity of *O. brevis* crude extracts and its fractions were examined against all the tested bacterial species using disc diffusion method of Kirby-Bauer technique[20]. Using cotton swabs, nutrient agar plates were uniformly inoculated with tryptic soy broth of bacterial cultures. A concentration of 10 mg mL^{-1} for each extract and fraction was prepared by dissolving 10 mg in 1 mL of dimethyl sulfoxide (DMSO). Sterilized discs (6 mm) from Whatman No. 1 filter paper were loaded by either extracts or fractions and dried completely under sterile conditions. The discs were placed on the seeded plates by using a sterile forceps. The DMSO and tetracycline (500 µg mL^{-1}) represented the negative control and positive control, respectively. Inoculated plates were incubated at 37°C for 24 h and then the inhibition zones were measured and expressed as the diameter of clear zone including the diameter of the paper disc.

Antifungal assay: The fungal strains were plated onto Potato Dextrose Agar (PDA) and incubated for 5 days at 25°C. The spore suspension (2×10^8 CFU mL^{-1}) of each fungus was prepared in 0.01% tween 80 solution by comparing with the 0.5 McFarland standard. Petri dishes containing YES medium were inoculated with 50 µL of each fungal culture and uniformly spread using sterile L-glass rod. Sterilized discs (6 mm) were loaded by either extracts or fractions (10 mg mL^{-1}) and dried completely under sterile conditions, then placed on the seeded plates by using a sterile forceps. The DMSO and commercial fungicide nystatin (1000 U mL^{-1}) were considered as a negative and positive control, respectively. The inoculated plates were incubated at 25°C for 48 h and then the antifungal activity was assessed by measuring the zone of inhibition (mm)[21]. The results average was calculated from at least three replicates for each assay.

***In vitro* cytotoxicity assay:** The *in vitro* cytotoxicity assay was conducted and assessed by the Bioassay-Cell Culture Laboratory, National Research Centre using the colorimetric method of Mosmann[22]. Three human cancer cell lines named hepatocellular carcinoma (HepG2), colon cancer (HCT116) and breast cancer (MCF7) were subjected to *O. brevis* extracts and its fractions. Cells were suspended in RPMI 1640 medium in 96-well microtiter plastic plates at concentration of 10×10^3 cells per well and kept at 37°C for 24 h under 5% CO_2 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 to give a final concentration of 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 4 h at 37°C 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°C. A positive control composed of Novantrone standard (100 µg mL^{-1}) was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions[23]. 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. The 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:

$$\text{Change in viability}(\%) = \left(\frac{\text{Reading of extract}}{\text{Reading of negative control}} - 1\right)\times 100 \quad (1)$$

A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

Fractionations of *O. brevis* crude extract using silica gel column chromatography: The Diethyl Ether Extracts (DEE) was fractionated using column chromatography technique. Glass column (30×500 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 per sec. 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.

GC/MS analysis: The diethyl ether fraction (F4) was 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°C. The oven temperature program was started at 50°C for 2 min. Then the temperature was ramped to150° at C7°C min^{-1}, then to 270°C at 5°C min^{-1} and held for 2 min, finally to 310°C at 3.5°C min^{-1} and held for 10 min. Mass Spectra were recorded under ionization energy of 70 eV.

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 the identified components was investigated using a percent relative peak area.

Statistical analysis: The experimental results were expressed as Mean±Standard Error (SE) for three replicates using SAS 6.03[24].

RESULTS AND DISCUSSION

Antimicrobial assay of *O. brevis* crude extracts: Eight extracts from *O. brevis* were evaluated for their potential biological activity against six pathogenic bacteria and nine mycotoxigenic fungi.

Antibacterial assay: The antibacterial activity of these extracts is illustrated in Table 1. The diameter of inhibition zone differed depending on the form of used solvent and the tested microorganism. The diethyl ether and chloroform extracts exhibited antibacterial activity against all the tested pathogenic bacteria; whereas aqueous extract had no antibacterial activity. Diethyl ether extract showed maximum antibacterial activity against both *E. coli* and *B. cereus* recording 32.0 mm inhibition zone.

No available studies examined the biological activity of *O. brevis* extract against pathogenic bacteria or mycotoxigenic fungi which considered one of the main food spoilage causes. The obtained results of antibacterial activity coincided with the study of Indumathi[25], who reported that *Oscillatoria* sp., diethyl ether extract had antibacterial activity against *E. coli*, *K. pneumoniae*, *Pseudomonas* sp., *Enterobacter* sp. and *S. typhi*. In contrast, Rath and Priyadarshani[26] reported that the diethyl ether extract *Oscillatoria* sp., had a lowest activities towards Gram+ve bacteria *B. subtilis* and *S. aureus* and moderate activities towards Gram-ve bacteria *P. aeruginosa* and *E. coli* whereas methanolic extract of *Oscillatoria* sp., gave the highest biological activity against *B. subtilis*, *S. aureus*, *P. aeruginosa* and *E. coli* followed by acetone. The present study also showed no inhibition effect of both acetone and aqueous extracts. However, Abd El-Aty *et al.*[27] found that all tested strains, *E. coli*, *S. aureus*, *S. typhi* and *P. aeruginosa* showed higher sensitivity to the acetone extract of *Oscillatoria agardhii*. Whereas the methanol extract showed moderate activity against all bacteria all species. They also reported the ineffectiveness of aqueous extracts against tested bacterial species except for *E. coli*. Other study showed that methanol, ethanol and chloroform extracts of *Oscillatoria sancta* like exhibited high activity against *E. coli*, *S. aureus*, *P. aeruginosa*, *S. typhi*, *B subtilis*, *K. pneumonia* and *C. albicans*[28]. Also, Selim *et al.*[29] found that *Oscillatoria* sp., ethanolic and methanol extracts had antibacterial activity, their effect varied depending on bacterial species. Ethanolic extract showed highest inhibition zone against *S. aureus*, *B. subtilis* and *E. coli*, respectively; while its methanolic extracts showed highest activity against *E. coli*, *S. aureus* and *B. subtilis*, respectively.

Antifungal assay: Table 2 illustrates the antifungal activity of *O. brevis* extracts against different species of mycotoxigenic fungi. In addition to the antibacterial activity, the diethyl ether extract showed antifungal activity against all tested fungal strains achieving inhibition zone ranged from 10.2-20.3 mm against *A. parasiticus* and *F. verticillioides*, respectively. *Aspergillus flavus* was the most sensitive fungus to all *O. brevis* extracts recording inhibition zone between 8.0 and 18.3 mm. However, *A. ochraceus* and *F. verticillioides* was tolerant of resistance to all *O. brevis* extracts with exception of diethyl ether extract.

None of the following studies examined algal extracts against broad wide of mycotoxigenic species. Khairy and El-Kassas[30] studied the antifungal activity of *Oscillatoria angustissima* ethyl acetate, chloroform, methanol, diethyl ether and aqueous extracts against *Aspergillus niger* and *Aspergillus flavus*. Only ethyl acetate and chloroform extract among these extracts had antifungal activity against *A. niger* and *A. flavus*. Also, Rath and Priyadarshani[26] reported that methanol, acetone and diethyl ether extracts of *O. boryana* and *Oscillatoria* sp., had antifungal activity against *A. niger*. Haggag *et al.*[31] reported that *O. agardhii* acetone, methanol and aqueous extracts had antifungal activity against mycotoxigenic fungi *Fusarium moniliforme*, *F. proliferatum*, *F. graminearum*, *Penicillium digitatum*, *Aspergillus niger* and *A. flavus*. Rajendran *et al.*[32] found that methanol, ethanol, chloroform and acetone extracts of *Oscillatoria* sp. had antifungal activity against *Fusarium* sp. The methanolic and ethanolic extracts showed higher antifungal activity, whereas the chloroform and acetone extracts showed moderate activity.

Table 1: Antibacterial activity of *Oscillatoria brevis* crude extracts

Bacteria	Inhibition zone (mm) Negative control	Positive control	Aqueous	MeOH	EtOH	Acetone	CH_3Cl	DEE	EtOA	Hexane
B. cereus	0	17.2±1.04	0	0	0	0	9.5±0.76	32.0±0.50	0	0
S. aureus	0	15.8±0.58	0	0	0	0	9.3±0.76	29.5±1.32	9.7±1.15	8.7±1.25
E. coli	0	13.2±1.08	0	0	7.8±1.04	0	10.0±0.50	32.0±1.32	0	7.8±1.04
S. typhi	0	19.2±1.26	0	0	8.0±0.86	0	9.0±0.50	28.0±1.32	9.3±1.52	8.2±0.28
P. aeruginosa	0	23.5±2.00	0	8.5±0.50	7.5±0.50	0	9.7±1.61	29.7±2.46	0	8.8±1.89
K. pneumonia	0	17.8±1.26	0	0	0	0	8.0±0.50	17.0±1.32	8.2±0.76	0

n = 3, SE: Standard error, 0: No inhibition, MeOH: Methanol, EtOH: Ethanol, DEE: Diethyl ether, EtOA: Ethyl acetate, negative control: DMSO, positive control: Tetracycline, Values are given as Mean±SE

Table 2: Antifungal activity of *Oscillatoria brevis* crude extracts

Fungi	Inhibition zone (mm) Negative control	Positive control	Aqueous	MeOH	EtOH	Acetone	CH_3Cl	DEE	EtOA	Hexane
A. flavus	0	14.1±0.58	11.8±1.75	8.8±0.76	8.5±1.32	12.0±1.29	8.0±0.50	18.3±0.76	11.2±1.25	9.5±1.50
A. steynii	0	9.3±0.35	8.8±0.76	7.2±0.28	8.8±1.44	7.8±1.32	0	14.3±1.61	8.2±1.04	0
A. ochraceus	0	11.2±0.64	0	0	0	0	0	10.3±1.32	0	0
A. parasiticus	0	11.8±1.11	0	0	0	8.2±0.76	11.5±1.32	10.2±1.04	0	0
A. westerdijikia	0	10.2±0.48	7.8±0.76	7.4±0.36	0	0	0	14.7±1.25	10.8±1.25	0
A. carbonarius	0	10.5±0.54	0	8.2±1.04	0	0	8.5±0.86	18.5±1.32	10.3±0.76	11.3±1.04
F. verticillioides	0	11.7±0.32	0	0	0	0	0	20.3±0.25	0	0
F. proliferatum	0	11.4±1.04	0	8.5±0.50	0	0	7.8±0.28	15.5±1.32	8.8±0.28	0
P. verrucosum	0	10.6±1.14	0	0	0	0	0	16.8±1.51	7.5±0.50	0

n = 3, SE: Standard error, 0: No inhibition, MeOH: Methanol, EtOH: Ethanol, DEE: Diethyl ether, EtOA: Ethyl acetate, negative control: DMSO, positive control: Nystatin, Values are given as Mean±SE

Antimicrobial assay of *O. brevis* diethyl ether fractions

Antibacterial assay: Based on the results of antibacterial and antifungal activities of *O. brevis*, diethyl ether extract was chosen to be fractionated using different elution formula. Seven fractions were separated to increase the probability of isolation and purification of certain compounds that responsible for the bioactivity. The antibacterial activity of *O. brevis* diethyl ether fractions is represented in Table 3. Both fraction F4 and F7 had antibacterial activity against all tested pathogenic bacteria; while F5 inhibited only *E. coli*. The highest antibacterial activity was shown using F7 against *S. typhi* with inhibition zone of 9.7 mm.

Many authors evaluated the effectiveness of diethyl ether extract of *Oscillatoria* species against bacteria. However none of them evaluate the chromatographic fractions of the crude extract, notably those of *O. brevis*. Shanab[33] reported that diethyl ether fractions of *O. rubescens*, *O. humilis* and *O. platensis* exhibited great antibacterial activity against *E. coli*, *B. subtilis* and *S. faecalis*. Madhumathi *et al.*[34] indicated that diethyl ether extract of *O. latevirns* had antibacterial activity against *B. subtilis*, *E. coli* and *S. mutans*, while *S. aureus* and *K. pneumonia* were resistance. Katircioglu *et al.*[35] found that ether extract of *Oscillatoria* sp., had antimicrobial activity against *B. subtilis*, *B. cereus*, *B. megaterium*, *E. coli*, *S. aureus* and *P. aeruginosa*. Also, Ahmadi and Hosseini[36] revealed that *Oscillatoria* sp., diethyl ether extract showed antibacterial activity against *E. coli* and *B. subtilis*. In contrast, Khairy and El-Kassas[30] reported that *O. angustissina* diethyl ether extract had not any antibacterial activity against *B. subtilis*, *B. cereus*, *S. aureus*, *E. coli* and *P. aeruginosa*.

Antifungal assay: The antifungal activity of *O. brevis* diethyl ether fractions against mycotoxigenic fungi are illustrated in Table 4. The only fraction had antifungal activity against all tested fungi was F4, whereas, F2 showed no antifungal activity against these fungi. *Aspergillus parasiticus* showed resistance against all fractions with exception of F4 which had 7.3 mm inhibition zone. The highest inhibition zone, 10.3 mm, was observed using F7 against *A. westerdijikia*.

Some studies reported the antifungal activity of diethyl ether extract of *Oscillatoria* species. Kim[37] reported that *O. angustissima* ether extract had antifungal activity against *F. oxysporum* and *Alternaria alternata*. Pawar and Puranik[38] indicated that *O. ornata* petroleum ether extract had antifungal activity against *A. niger* and *F. oxysporum*. In contrast, Shanab[33] found that diethyl ether fractions of *O. rubescens*, *O. humelli* and *O. platensis* showed no

Table 3: Antibacterial activity of *Oscillatoria brevis* diethyl ether fractions

	Inhibition zone (mm)							
Bacteria	control	F1	F2	F3	F4	F5	F6	F7
B. cereus	0	0	0	0	8.3±1.15	0	0	7.0±0.15
S. aureus	0	9.0±1.73	0	0	7.3±0.32	0	0	7.7±0.58
E. coli	0	0	8.3±1.04	8.33±1.15	7.0±0.28	7.0±0.15	9.3±0.58	9.0±0.28
P. aeruginosa	0	0	0	0	7.3±1.15	0	0	7.7±1.00
S. typhi	0	8.7±0.58	0	0	8.7±0.28	0	0	9.7±0.58
K. pneumonia	0	7.7±0.58	8.0±1.00	8.0±1.00	7.7±0.58	0	8.7±0.58	8.0±0.15

n = 3, SE: Standard error, 0: No inhibition, control: DMSO, Values are given as Mean±SE

Table 4: Antifungal activity of *Oscillatoria brevis* diethyl ether fractions

	Inhibition zone (mm)							
Fungi	Control	F1	F2	F3	F4	F5	F6	F7
A. flavus	0	0	0	0	7.7±0.58	7.3±0.28	0	6.8±0.28
A. steynii	0	7.7±1.15	0	0	8.7±1.52	0	7.3±0.58	8.0±1.00
A. ochraceus	0	0	0	0	10.0±1.73	0	10.3±0.58	0
A. parasiticus	0	0	0	0	7.3±0.58	0	0	0
A. westerdijikia	0	8.3±1.04	0	9.7±1.52	7.3±0.58	0	0	10.3±0.58
A. carbonarius	0	8.0±1.04	0	0	9.3±1.08	0	9.0±0.00	9.0±1.00
F. verticillioides	0	8.0±1.00	0	0	7.3±0.58	8.0±1.00	0	0
F. proliferatum	0	7.7±1.15	0	0	7.0±0.00	0	0	0
P. verrucosum	0	7.3±0.58	0	0	7.3±0.28	0	0	0

n = 3, SE: Standard error, 0: No inhibition, control: DMSO, Values are given as Mean±SE

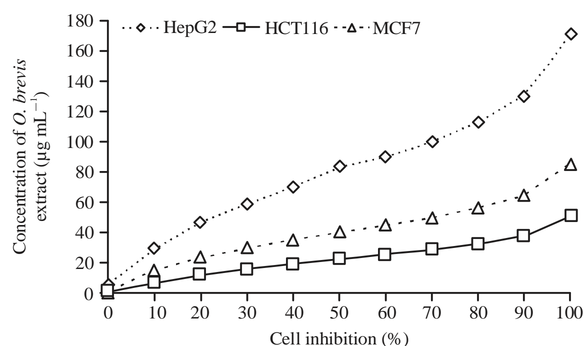

Fig. 1: *In vitro* cytotoxicity assay of *O. brevis* diethyl ether crude extract using HePG2, HCT116 and MCF7 cell lines

antifungal activity against *A. flavus*. Also, Padhi *et al.*[39] reported that *O. princeps* ether extract had not antifungal activity against *P. notatum*, *F. moniliforme* and *A. niger* while benzene extract had bioactivity against these fungi.

***In vitro* cytotoxicity of *O. brevis* diethyl ether extract:** The cytotoxicity of *O. brevis* diethyl ether extract against HePG2, HCT116 and MCF7 cell lines is represented in Fig. 1. Small concentration of ether extract showed high inhibition against HCT116 and MCF7 cell lines at IC_{50} of 22.0 and 39.7 μg mL^{-1}, respectively, while moderate anticancer bioactivity was illustrated against HePG2 cell lines with IC_{50} of 83.4 μg mL^{-1}.

Mevers *et al.*[40] reported that the methanolic extract of *Oscillatoria terebriformis* had cytotoxicity effect against A549 lung cancer cells with LC_{50} of 31.25 μg mL^{-1}. Shanab *et al.*[41] revealed that *Oscillatoria* sp., aqueous extract recorded high anticancer activity 77.8% against liver cancer cell line HepG2 at 100 μg mL^{-1}. In another study, Nair and Bhimba[42] reported that *Oscillatoria boryana* ethanolic extract showed anticancer activity against human breast cancer cell lines MCF7 with LC_{50} of just 10.45 μg mL^{-1}. However, Maruthanayagam *et al.*[43] reported that the concentration 10 μg m^{-1} of the mixture of chloroform: methanol (1:1) extract of *Oscillatoria* sp., *O. formosa*, *O. laetevirens* and *O. salina* showed no activity against HT29 colon, HoP62 lung, MCF7 breast and KB oral cell line.

***In vitro* cytotoxicity of *O. brevis* diethyl ether fractions:** Since, fraction F4 of *O. brevis* had the highest activity as antimicrobial agent against tested bacteria and fungi. This fraction was examined for their cytotoxic activity against HepG2, HCT116 and MCF7 cancer cell lines. The cytotoxicity of *O. brevis* fraction F4 is illustrated in Fig. 2. The highest cytotoxic activity was recorded against breast cancer cell lines MCF7 with IC_{50} of 20.6 μg mg^{-1}, followed by colon cancer cell lines HCT116 with IC_{50} of 23.4 μg mL^{-1}. While, no anticancer activity of *O. brevis* fraction F4 was showed against liver cancer cell lines HepG2 by using concentrations reach to 250 μg mL^{-1}.

The cytotoxicity of F4 at IC_{50} (20.6 μg mg^{-1}) was doubled against MCF7 and did not change against HCT116 when compared with that of diethyl ether crude extract. From this observation, it was revealed that F4 contained most of compounds responsible of its activity against MCF7.

Table 5: Chemical constituents of *O. brevis* fraction F4 detected by GC/MS

RT	Compounds	Area (%)
17.38	Phenol, 2,4-bis(1,1- dimethylethyl)	12.83
20.22	Heptadecane, 7-methyl	3.47
27.00	Pentadecanoic acid, 14-methyl-, methyl ester	6.18
29.83	Decane, 2,6,6-trimethyl	29.84
30.35	9,12-octadecadienoic acid, methyl ester	7.09
31.45	9-octadecadienoic acid (Z)-methyl ester	10.36
37.77	Pentacosane (CAS)	3.34
50.07	2,4-dimethyl-2-nitro-(CAS)	2.87
56.82	Quercetin 7,3',4'-trimethoxy	1.88
58.89	Octasiloxane	1.08

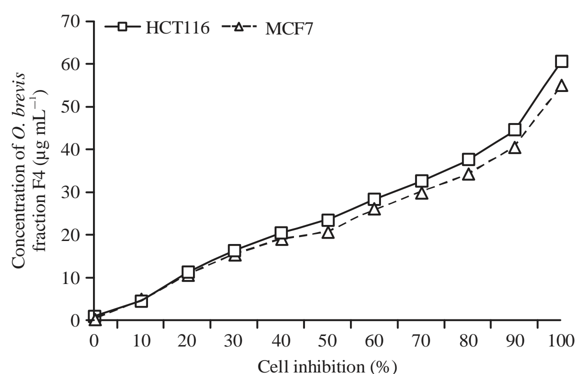

Fig. 2: *In vitro* cytotoxicity assay of *O. brevis* diethyl ether fraction-4 (F4) using HCT116 and MCF7 cell lines

The cytotoxic activity may be attributed to presence of bioactive compounds in the fractions. Roussis *et al.*[44] reported that the lipophilic fractions of *Q. acutissima* had anticancer activity against colon cancer cell lines HCT116 and breast cancer cell lines MCF7 with LC_{50} of 9.5 and 6.0 µg mL^{-1}, respectively. Likewise, Shanab *et al.*[41] found that major secondary metabolites of *Oscillatoria* sp., total phenolic content, terpenoids and alkaloids as well as phycobiliprotein pigments, phycocyanin, allophycocyanin and phycoerythrin were showed to have anticancer activity against Ehrlich Ascites Carcinoma Cell (EACC) and Human hepatocellular cancer cell line (HepG2).

GC/MS analysis of fraction (F4): The results pertaining to GC/MS analysis of *O. brevis* fraction (F4) are illustrated in Table 5. It shows 10 compounds with retention time ranging from 17.38-58.89 min. The maximum peak was identified as 2, 6, 6-trimethyl-Decane (29.84%) followed by 2-phenyl-4-trimethylsilyl-3-buten-2-ol (12.83%), while, the minimum peak was identified as quercetin 7, 3', 4'-trimethoxy (1.87%) and octasiloxane (1.08%).

Most of the identified compounds have been reported to possess biological activities properties. The fatty acids 9-octadecenoic acid and 9,12-octadecadienoic acid detected in the *O. brevis* fraction (F4) have been previously isolated from microalgae *Nannochloropsis oculata* and they exhibited antibacterial activity against *P. aeruginosa*, *E. coli*, *B. subtilis* and *S. aureus*[45]. These fatty acids have been detected in the methanol extract of green alga *Spirogyra rhizoids* and they showed antimicrobial activity against *B. cereus*, *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. typhi*, *S. faecalis*, *S. pyogenes*, *V. cholerae*, *F. oxysporum* and *A. flavus*[46], while Kumar *et al.*[47], Jain *et al.*[48] and Govindappa *et al.*[49] isolated these fatty acids from some plants and *Spirulina platensis* display antibacterial and antifungal activity against several human pathogenic microorganisms. Also, the following compounds, octasiloxane, phenol, 2, 4-bis (1, 1-dimethylethyl) and pentadecanoic acid, 14-methyl-, methyl ester have been detected in the *O. brevis* fraction (F4). These compounds were isolated and reported to have antibacterial and antifungal activity[48,50,51].

The compounds, phenol, 2,4-bis(1,1- dimethylethyl) and pentadecanoic acid, 14-methyl-, methyl ester, detected in the current study were exhibited antibacterial, antifungal and antioxidant activities[52-55]. Salem *et al.*[56] reported that quercetin 7,3',4'-trimethoxy from *Nostoc* sp., methanol extract had antimicrobial activity against *B. subtilis*, *K. pneumonia*, *S. aureus* and *A. niger*.

Cytotoxicity of the *O. brevis* fraction-4 (F4) was attributed to some chemical constituents detected in the GC/MS analysis such Phenol, 2,4-bis(1,1-dimethylethyl)-, 9,12-Octadecadienoic acid, methyl ester and Quercetin, where these compound have been reported to have an anticancer activity against MCF7 cell line[57,58]. Also, phenol, 2,4-bis(1,1-dimethylethyl) and quercetin exhibited moderate activity against HCT116 cell line[58,59]. These compounds may work individually or work synergistically. Quercetin can inhibit the growth of several human cancer cell lines by preventing oxidative DNA damage. Although, Qu exerted high cytotoxicity on cancer cells, it showed no damages for normal cells[60].

CONCLUSION

Cyanobacterium, *Oscillatoria brevis*, can be considered as a novel source of bioactive metabolites. Diethyl ether crude extract of *O. brevis* was the one had the greatest antimicrobial and anticancer activity. By fractionation, F4 effectively inhibited all pathogenic bacteria and mycotoxigenic fungi. Ten compounds were identified in F4, most of them have been proved to possess biological activities against different pathogenic bacteria, mycotoxigenic fungi and Human cancer cell line. Finally, the findings of the present study will serve as a base for future studies to develop food preservative and nutraceutical agents from algal natural sources.

SIGNIFICANCE STATEMENTS

This study considers the first one in Egypt evaluated the antibacterial, antifungal and anticancer activities of *Oscillatoria brevis* extracts and its fractions. It discovered few compounds known to have cytotoxicity against breast cancer. So, this study will help bio-preservative and nutraceutical industries basing on algal natural sources.

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