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Antimicrobial Activity, Cytotoxicity and Chemical Constituents of the Freshwater Microalga *Oscillatoria princeps*

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Abstract: The microalgae *Oscillatoria* sp. are promising sources of bioactive metabolites used in both pharmaceutical and nutraceutical applications. The main objective of present study is to determine the antimicrobial activity, cytotoxicity, and chemical profile of *Oscillatoria princeps* r extracts and its fractions. *O. princeps* extracts were prepared by successive extraction method, and chemical constituents were identified using GC-MS. Diethyl ether extract (DEE) had antimicrobial activity against all tested microorganisms and the highest inhibition zones 20.7 and 20.2 mm was observed against *P. aeruginosa* and *A. flavus*, respectively. Also, DEE showed an anticancer activity with IC_{50} values of 35.18, 46.6, and 79.18 µg ml^{-1} against breast cancer (MCF7), colon cancer (HCT116), and hepatocellular carcinoma (HePG2) cell lines, respectively. By DEE fractionation, fraction F7 showed the highest antimicrobial activity followed by fraction F4 with minimum inhibitory concentration (MIC) values ranged between 0.5 and 1.9 mg ml^{-1}. Fraction F4 recorded anticancer activity against HCT116, MCF7, and HePG2 with IC_{50} of 22.62, 24.43, and 102.52 µg ml^{-1}, respectively. While fraction F7 had anticancer activity against HCT116 and MCF7 without any effect on the HePG2 cell line. GC-MS analysis of fractions F4 and F7 represented that the main compounds responsible for the bioactivity were Pentadeconic acid,4-hexadecyl ester, and 9-Octadecenoic acid in F4, while the main compounds in F7 were Quercetin 7,3',4'-trimethoxy and Methyl tetradecanoate. The study concluded that *O. princeps* DEE extract and fractions had a sufficient amount of bioactive compounds that possess antimicrobial and anticancer activity, which could be a promising source for pharmaceutical and nutraceutical ingredients.

Keywords: *Oscillatoria princeps*; antimicrobial activity; cytotoxicity; chemical constituents.

1. Introduction

Natural products are the main source for drug discovery and development. Many microorganisms and plants have a great attraction as a natural source of bioactive molecules [1–6]. Blue-green algae have become an important target for pharmaceutical and biotechnology industries because of the huge number of bioactive compounds with a wide range of biological activities discovered from them [7-11]. Cyanobacteria are prokaryotic forms that use chlorophyll for photosynthesis, therefore many species capable of nitrogen fixation. They can also grow under heterotrophic conditions using the organic carbon substrates as energy sources

[12-16]. Cyanobacteria are found in many different habitats, from fresh to marine, hyper-saline, and terrestrial ecosystems [17, 18].

Oscillatoriaceae is one of the most important orders in blue-green algae. More than 15 species are present in Oscillatoriaceae order like *Spirulina*, *Oscillatoria*, *Phormidium*, and*Lyngbya*. Oscillatoriaceae species produce many forms of biologically and interesting significant bioactive metabolites (more than 300 compounds are reported), which cover almost all of these biological activities [19-21]. *Oscillatoria* species produce many pharmaceutical and nutraceutical ingredients with varying bioactivities, including antibacterial, antifungal, antioxidant, antialgal, antiviral, anticancer, and anti-inflammatory effects [22]. These bioactive ingredients ranged between phenolic compounds, alkaloids, fatty acid, pigments and pigments derivatives, linear and cyclic peptides, terpenoids, and N-glycosides [23-27]. The present study aimed to determine the antimicrobial activity, cytotoxicity, and chemical profile of *Oscillatoria princeps* diethyl ether extract (DEE) and its fractions. The present study aimed to observe the pharmaceutical, food preservation, and nutraceutical activity of *Oscillatoria princeps* extracts by evaluating the antimicrobial and anticancer activities of these extracts and their fractions. Followed by the identification of the chemical profile of bioactive fractions using GC-MS.

2. Materials and Methods

2.1. Microalgae strain and culture medium.

A pure isolate of *Oscillatoria princeps* was obtained from Marine Toxins Lab., National Research Centre, Egypt [28]. The culture medium used for cultivation was BG-11 [29]. At the maximum growth phase, 21 days, *O. princeps* biomass was harvested and dried overnight in a hot air oven at 50 ºC.

2.2. Preparation of O. princeps extracts.

The dried *O. princeps* biomass (100 g) was successive extraction using several solvents according to the polarity (hexane, diethyl ether, chloroform, acetone, ethyl acetate, ethanol, methanol, and deionized water). Each homogenized dried biomass was sonicated for 20 min using an ultrasonic micro tip probe of 400 watts (ULTRASONIC Get 750), then centrifuged at 4500 rpm for 10 min (SIGMA Laborzentrifugen Gmbh). Supernatants were collected separately, and the pellets were re-extracted twice, as mentioned before. Combined supernatants were evaporated to dryness at 40ºC using a rotary evaporator. Dried extracts were kept in labeled sterile vials in a deep freezer at -20 ºC till further use [7].

2.3. Fractionations of O. princeps crude extract.

The diethyl ether extracts (DEE) was fractionated using column chromatography technique. Glass column (30 x 500 mm) was initially packed with 5 g of anhydrous sodium sulfate followed by 30 g of silica gel (0.06 - 0.2 mm, 70 - 230 mesh ASTM) using chloroform as a carrier solvent to create a slurry. Finally, 5 g of anhydrous sodium sulfate was added to the silica gel's top to prevent the 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 [19].

2.4. GC/MS analysis of O. princeps bioactive fractions.

The diethyl ether fraction F4 and F7 were subjected to analysis of chemical composition using GC/MS, Thermo Scientific, Trace GC Ultra coupled with ISQ Single Quadrupole mass spectrometer (MS). Components were separated 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 [30]. Tentative identification of the compounds was performed based on comparing 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.

2.5. Antimicrobial activity of O. princeps crude extract and fractions.

2.5.1. Test microorganisms.

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

2.5.2. Disc diffusion assay.

From the 24 h incubated nutrient agar slant of each bacterial strain, a full loop of the bacteria was inoculated in a tube containing 5 ml of tryptic soy broth. The broth culture was incubated at 35ºC for 2-6 h until it achieves the turbidity of 0.5 McFarland $BaSO_4$ standard. The inhibitory effect of *O. princeps* crude extracts and its fractions were tested against all the tested bacterial species using the disc diffusion method of Kirby-Bauer technique [31-33]. A concentration of 10 mg ml^{-1} for each extract and fraction was in 1 ml of dimethyl sulfoxide (DMSO). DMSO represented the negative control, and tetracycline (500 mg ml^{-1}) was used as a positive control. The inoculated plates were incubated at 37 ºC for 24 h. After incubation, the inhibition zones were measured and expressed as the clear zone's diameter, including the diameter of the paper disc.

The fungal strains were plated onto potato dextrose agar (PDA) and incubated for 5 days at 25 ºC. The spore suspension (2 $x10^8$ cfu ml^{-1}) of each fungus was prepared in 0.01% Tween 80 solution by comparing with the 0.5 McFarland standard. Commercial fungicide Nystatin (1000 Unit ml^{-1}) and DMSO considered positive and negative control, respectively. The inoculated plates were incubated at 25ºC for 48 h, then the antifungal activity was assessed by measuring the zone of inhibition (mm) [34]. The results average was calculated from at least three replicates for each assay.

2.5.3. Determination of minimum inhibitory concentration (MIC).

The determination of MIC was conducted using the micro broth dilution method as of Andrews [35]. Two-fold serial dilutions of *O. princeps* ether extract and fractions with nine different concentrations of each *O. princeps* ether extract and fractions (4.0, 2.0, 1.75, 1.5, 1.0, 0.75, 0.50, 0.25, 0.1 mg ml^{-1} in DMSO) were prepared. Each tube was inoculated with 100 ml of bacterial cell suspension and incubated at 37 ºC for 24 h. Equal volumes of tested bacteria (10^5 cfu ml^{-1}) were added to each well. MIC values were taken as the lowest concentration of the antimicrobial agent that inhibited bacterial growth after 24 h incubation at 37 °C.

MIC against fungi was performed by using the technique of Perrucci *et al*. [36]. Crude extracts and fractions at different concentrations were separately dissolved in 0.5 ml of 0.1% Tween 80, then mixed with 9.5 ml of melting, 45 ºC, PDA, and poured into Petri dish (6 cm). The prepared plates were centrally inoculated with 3 ml of fungal suspension (10^8 cfu ml^{-1}; 0.5 McFarland standard). The plates were incubated at 25 ºC for 24-48 h. At the end of the incubation period, mycelial growth was monitored, and MIC was determined.

2.6. In vitro cytotoxicity assay of O. princeps ether extract and fractions.

The cytotoxic activity test (*In vitro assay*) on three human cancer cell lines, colon cancer (HCT116), hepatocellular carcinoma (HepG2), and breast cancer (MCF7) were conducted and assessed by the Bioassay-Cell Culture Laboratory, National Research Centre. The cytotoxicity evaluation of *O. princeps* extracts and their fractions was examined using the colorimetric method of Mosmann [37]. Cell proliferation assay was performed via 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT). MTT (0.5 mg ml^{-1}) was prepared, and MTT solution was added to each of the 96-well microtiter plastic plates. The viability of cancer cells was assessed by reducing the yellow dye (MTT) to purple formazan product. 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 the vehicle) using the dependent t - test by the SPSS 11 program. DMSO is the vehicle used to dissolve 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:

$$\{(\text{Reading of extract} / \text{Reading of negative control}) - 1\} \times 100$$

Probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 16.0 program.

2.7. Statistical analysis.

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

3. Results and Discussion

3.1. Antimicrobial activity of O. princeps crude extracts.

Table 1 illustrates the antibacterial activity of *O. princeps* extracts against different species of foodborne pathogenic bacteria. It is clear that the diameter of the inhibition zone

depends on the form of solvent used and the tested pathogenic bacteria. The diethyl ether extracts of *O. princeps* showed the highest antibacterial activity against *P. aeruginosa* and *B. cereus*, which recorded 20.7 and 20.2 mm inhibition zones, respectively. Diethyl ether extract has antibacterial activity against all tested pathogenic bacteria, followed by ethanol extract, which has antibacterial activity against all tested bacteria except *K. pneumonia*, and chloroform extract, which has antibacterial activity against all tested bacteria except *S. typhi*. While aqueous extract has no activity against all tested bacteria, followed by methanol extract, which has antibacterial activity only against *K. pneumonia*, and acetone extract, which has antibacterial activity against *S. aureus* and *B. cereus*.

The antifungal activity of *O. princeps* extracts against different mycotoxigenic fungi species is shown in Table 2. The diethyl ether extracts of *O. princeps* showed the best antifungal activity against all tested fungi achieving an inhibition zone in a range between 7.3 mm against *P. verrucosum* to 15.7 mm against *A. westerdijikia*. *A. flavus* was the most sensitive fungus affected by all extracts, recording an inhibition zone between 9.7 and 14.3 mm. However, *A. ochraceus* was tolerant of resistance to all *O. princeps* extracts except diethyl ether extract, which had an inhibition zone of 7.7 mm.

Mathivanan *et al*. [38] found that *O. princeps* ethanol, acetone, methanol, and aqueous extracts had antibacterial activity against *S. aureus*, *P. aeruginosa*, and *B. subtilis* being the ethanolic extract as the best. Al-Rubaiee and Shaukat [39] reported that *O. princeps* extract of mixture chloroform: methanol (2:1) had antibacterial activity against *S. aureus*, *B. subtilis*, *K. pneumonia*, *P. aeruginosa*, and *E. coli*. They found that the highest antibacterial activity was against *S. aureus* with an inhibition zone of 30mm, followed by *B. subtilis* and *P. aeruginosa* with 28 and 25 mm inhibition zone, respectively. Marrez *et al*. [19] revealed that *Oscillatoria brevis* chloroform and diethyl ether extracts had antibacterial activity against *B. subtilis*, *S. aureus*, *E. coli*, *S. typhi*, *P. aeruginosa* and *K. pneumoniae* with a zone of inhibition ranged between 8 and 32 mm. Also, Rath and Priyadarshan [40] reported that the diethyl ether extract *Oscillatoria* sp. had the least 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.

Abd El-Aty *et al*. [41] reported that all tested strains, *E. coli*, *S. aureus*, *S. typhi*, and *P. aeruginosa* showed higher sensitivity acetone extract of *Oscillatoria agardhii*. At the same time, 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*. Selim *et al*. [42] found that *Oscillatoria* sp. ethanolic and methanol extracts had antibacterial activity; their effect varied depending on bacterial species. Ethanolic extract showed the highest inhibition zone against *S. aureus*, *B. subtilis*, and *E. coli*, respectively, while its methanolic extracts showed the highest activity against *E. coli*, *S. aureus*, and *B. subtilis*, respectively. Khairy and El-Kassas [43] studied the Antifungal activity of *Oscillatoria angustissima* ethyl acetate, chloroform, methanol, diethyl ether aqueous extracts against *Aspergillus niger* and *Aspergillus flavus*. Only ethyl acetate and chloroform extract among these extracts have antifungal activity against *A. niger* and *A. flavus*. Few studies examined the antifungal activity of *O. princeps* extracts.

Table 1. Antibacterial activity of *Oscillatoria princeps* crude extracts.

Bacteria	Inhibition zone mm (Mean±*S.E)									
	-ve control	+ve control	Aqueous	MeOH	EtOH	Acetone	CH_3Cl	DEE	EtOA	Hexane
B. cereus	0	16.8 ± 0.86^{b}	0	0	7.8 ± 1.04^{d}	8.0 ± 0.50^{d}	9.3 ± 0.58^{c}	20.2 ± 0.76^{a}	8.3 ± 0.29^{d}	0
S. aureus	0	17.2 ± 1.04^{b}	0	0	7.3 ± 0.28^{d}	8.0 ± 1.15^{d}	7.3 ± 0.58^{d}	19.5 ± 2.18^{a}	9.8 ± 1.04^{c}	9.2 ± 1.04^{c}
E. coli	0	18.6 ± 1.28^{a}	0	0	10.0 ± 1.29^{b}	0	8.7 ± 1.04^{bc}	19.0 ± 1.32^{a}	9.0 ± 0.32^{bc}	8.5 ± 0.5^{c}
S. typhi	0	15.5 ± 1.00^{b}	0	0	8.7 ± 0.58^{c}	0	0	17.3 ± 1.25^{a}	0	0
P. aeruginosa	0	19.8 ± 1.14^{a}	0	0	8.8 ± 1.25^{c}	0	9.7 ± 0.28^{b}	20.7 ± 2.08^{a}	9.7 ± 1.44^{b}	8.3 ± 1.15^{c}
K. pneumonia	0	18.6 ± 1.58^{a}	0	8.5 ± 0.50^{d}	0	0	10.0 ± 0.86^{c}	14.7 ± 1.25^{b}	0	0

n=3, *S.E: standard error, 0: No inhibition, -ve control: DMSO, +ve control: tetracycline, MeOH: methanol, EtOH: ethanol, DEE: diethyl ether, EtOA: ethyl acetate, different superscripts within row are significantly different at the 5% level.

Table 2. Antifungal activity of *Oscillatoria princeps* crude extracts.

Fungi	Inhibition zone mm (Mean±*S.E)									
	-ve control	+ve control	Aqueous	MeOH	EtOH	Acetone	CH_3Cl	DEE	EtOA	Hexane
A. flavus	0	15.8 ± 0.58^{a}	12.0 ± 1.04^{b}	10.7 ± 0.75^{c}	10.3 ± 1.04^{c}	10.2 ± 1.50^{c}	12.2 ± 0.76^{b}	14.3 ± 2.29^{ab}	9.8 ± 1.25^{c}	9.7 ± 0.58^{c}
A. niger	0	8.5 ± 0.36^{c}	0	9.0 ± 1.29^{c}	0	10.2 ± 125^{b}	11.3 ± 1.04^{a}	10.5 ± 1.32^{b}	9.7 ± 1.15^{b}	7.8 ± 0.28^{d}
A. ochraceus	0	10.8 ± 0.76^{a}	0	0	0	0	0	7.7 ± 1.25^{b}	0	0
A. parasiticus	0	11.8 ± 1.04^{b}	0	0	0	0	9.8 ± 1.04^{c}	14.8 ± 0.76^{a}	8.5 ± 0.86^{d}	0
A. westerdijikia	0	10.8 ± 0.86^{b}	9.5 ± 0.86^{c}	0	8.5 ± 0.87^{d}	0	0	15.7 ± 1.61^{a}	8.0 ± 0.86^{d}	0
A. carbonarus	0	10.5 ± 0.50^{a}	0	8.7 ± 0.28^{b}	0	0	0	11.3 ± 0.76^{a}	9.2 ± 0.76^{b}	0
F. verticelloides	0	11.2 ± 0.68^{b}	7.8 ± 0.28^{c}	8.3 ± 1.04^{c}	0	0	0	13.5 ± 0.60^{a}	0	11.7 ± 0.76^{b}
F. proleferatum	0	10.9 ± 0.58^{b}	0	0	0	9.8 ± 1.15^{c}	9.2 ± 1.04^{c}	15.0 ± 1.50^{a}	0	0
P. verrucosum	0	10.1 ± 1.14^{a}	0	8.5 ± 0.86^{b}	7.7 ± 0.58^{c}	0	0	7.3 ± 0.58^{c}	0	0

n=3, *S.E: standard error, 0: No inhibition, -ve control: DMSO, +ve control: Nystatin, MeOH: methanol, EtOH: ethanol, DEE: diethyl ether, EtOA: ethyl acetate, different superscripts within row are significantly different at the 5% level.

Mathivanan *et al*. [38] found that *O. princeps* ethanol, acetone, methanol, and aqueous extracts have antifungal activity against *A. niger*. However, they reported that the ethanolic extract showed the highest inhibition zone, 14 mm. No inhibition was observed in the current study using ethanol extract against *A. niger*.

Also, Rath and Priyadarshanl [40] reported that methanol, acetone and diethyl ether extracts of *O. boryana* and *Oscillatoria* sp. have antifungal activity against *A. niger*. Haggag *et al*. [44] r eported 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*. [45] 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. Marrez *et al*. [19] examined the antifungal activity of several *O. brevis* extracts and found that diethyl ether extract had the highest antifungal activity against *F. proliferatum, F. verticillioides, P. verrucosum, A. flavus, A. steynii, A. ochraceus, A. parasiticus, A. westerdijikia, A. carbonarius*.*3.2. Cytotoxic activity of O. princeps diethyl ether extract.*

The cytotoxicity of *O. princeps* diethyl ether extract against HePG2, HCT116, and MCF7 cell lines is represented in Fig. 1. A small concentration of ether extract, showed high inhibition against MCF7 and HCT116 cell lines at IC_{50} of 35.18 and 46.6 µg ml^{-1}, respectively, while moderate anticancer bioactivity was illustrated against HePG2 cell lines with IC_{50} of 79.18 µg ml^{-1}.

Mevers *et al*. [46] reported that the methanolic extract of *Oscillatoria terebriformis* had a cytotoxicity effect against A549 lung cancer cells with IC_{50} of 31.25 µg ml^{-1}. Shanab *et al*. [47] revealed that *Oscillatoria* sp. aqueous extract recorded high anticancer activity 77.8% against liver cancer cell line HepG2 at 100 µg ml^{-1}. Maruthanayagam *et al*. [48] found that the mixture of chloroform: methanol (1:1) extract of *Oscillatoria* sp., *O. formosa*, *O. laetevirens*, and *O. salina* showed moderate cytotoxicity with IC_{50} values from 167 to 325 µg ml^{-1} against *Artemia salina*. In contrast, they found that these extracts have not any activity against HT29 colon, HoP62 lung, MCF7 breast, and KB oral cell line at concentration reach to 10 µg ml^{-1}. Also, Nair and Bhimba [49] reported that *Oscillatoria boryana* ethanolic extract showed anticancer activity against human breast cancer cell lines MCF7 with IC_{50} of 10.45 µg ml^{-1}. Mukund *et al*. [50] indicated that *Oscillatoria margaritifera* dichloromethane and methanolic extracts affected lung cancer cell lines with IC_{50} of 0.14 µM. Marrez *et al*. [19] indicated that *Oscillatoria brevis* diethylether extracts had a high inhibitory effect against HCT116 and MCF7 cell lines with IC_{50} values of 22 and 39.7 µg ml^{-1}, respectively and moderated anticancer activity against HePG2 cell line with IC_{50} value 83.4 µg ml^{-1}.

3.3. Antimicrobial activity O. princeps diethyl ether fractions.

The antibacterial activity of *O. princeps* diethyl ether fractions is represented in Table 3. Fraction F7 had antibacterial activity against all tested foodborne pathogenic bacteria followed by F4, which had bioactivity against all tested bacteria except *B. cereus* and *K. pneumonia*, while F3 had antibacterial activity only against *S. aureus* and *S. typhi*. In contrast, F1, F2, F5, and F6 showed no antibacterial activity against all tested bacteria. The highest antibacterial activity was showed using F7 against *S. aureus* and *P. aeruginosa* with an inhibition zone of 9.7 mm.

Table 4 illustrates the antifungal activity of *O. princeps* against toxic fungi. No fraction had antifungal activity against all tested fungi. Fraction F7 showed antifungal activity against all tested fungi except *A. flavus*, followed by F4, which had activity against all tested fungi except *A. parasiticus* and *P. verrucosum*. Whereas F1 and F2 showed no antifungal activity against all tested fungi. The highest inhibition zone, 9.3 mm, was observed using F4 against *A. carbonarus*.

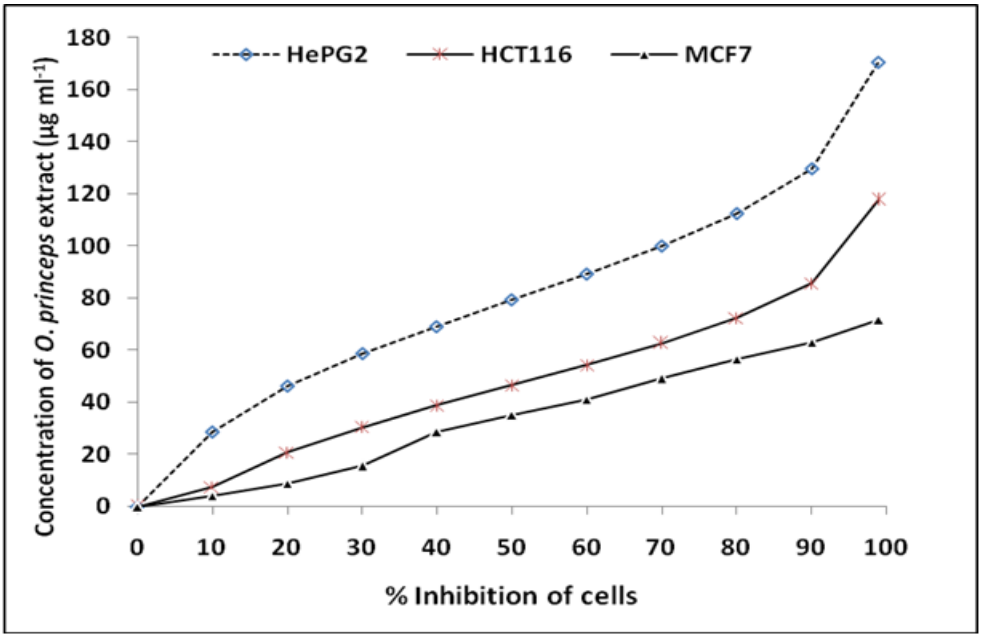

Figure 1. Cytotoxicity of *O. princeps* diethyl ether extract on HePG2, HCT116, and MCF7 cell lines.

Table 3. Antibacterial activity of *Oscillatoria princeps* DEE extracts fractions.

Bacteria	Inhibition zone mm (Mean±*S.E)								
	-ve control	-ve control	F1	F2	F3	F4	F5	F6	F7
B. cereus	0	16.5±0.50^{a}	0	0	0	0	0	0	9.0±1.15^{b}
S. aureus	0	17.0±1.00^{a}	0	0	8.3±1.52^{c}	7.7±1.15^{d}	0	0	9.7±0.58^{b}
E. coli	0	18.8±1.14^{a}	0	0	0	8.0±1.00^{b}	0	0	8.0±0.00^{b}
S. typhi	0	15.2±0.86^{a}	0	0	7.7±0.58^{c}	8.0±1.00^{c}	0	0	9.0±1.73^{b}
P. aeruginosa	0	19.5±1.08^{a}	0	0	0	7.7±1.15^{c}	0	0	9.7±1.88^{b}
K. pneumonia	0	18.4±1.28^{a}	0	0	0	0	0	0	7.3±0.58^{b}

n=3, *S.E: standard error, 0: No inhibition, -ve control: DMSO, +ve control: tetracycline, different superscripts within row are significantly different at the 5% level.

Table 4. Antifungal activity of *Oscillatoria princeps* DEE extracts fractions.

Fungi	Inhibition zone mm (Mean±*S.E)								
	-ve control	-ve control	F1	F2	F3	F4	F5	F6	F7
A. flavus	0	15.3±0.48^{a}	0	0	7.3±0.28^{c}	8.3±0.28^{b}	0	7.2±0.76^{c}	0
A. niger	0	8.5±0.36^{c}	0	0	0	7.7±1.15^{b}	0	8.0±1.73^{b}	7.7±1.15^{b}
A. ochraceus	0	10.8±0.76^{a}	0	0	0	9.0±2.00^{b}	8.0±1.00^{c}	7.7±1.15^{c}	8.0±1.73^{c}
A. parasiticus	0	11.8±1.04^{b}	0	0	7.3±0.28^{b}	--	7.7±0.58^{b}	0	8.0±1.00^{b}
A. westerdijikia	0	10.8±0.86^{a}	0	0	0	9.0±1.00^{b}	0	0	8.3±0.58^{b}
A. carbonarus	0	10.6±0.36^{a}	0	0	0	9.3±1.52^{b}	8.0±1.00^{c}	7.7±1.15^{c}	8.0±1.73^{c}
F. verticelloides	0	11.4±0.86^{a}	0	0	7.7±1.15^{c}	8.3±0.58^{bc}	0	7.3±0.58^{c}	9.0±1.00^{b}
F. proliferatum	0	11.2±0.48^{a}	0	0	8.0±1.00^{b}	7.7±0.58^{b}	0	0	7.3±0.58^{c}
P. verrucosum	0	10.6±1.04^{a}	0	0	7.3±0.58^{b}	0	0	0	7.7±0.28^{b}

n=3, *S.E: standard error, 0: No inhibition, -ve control: DMSO, +ve control: Nystatin, different superscripts within row are significantly different at the 5% level.

Shanab [51] reported that diethyl ether fractions of *O. rubescens*, *O. humelli*, and *O. platensis* exhibited great antibacterial activity against *E. coli*, *B. subtilis*, *S. oblus*, and *S. faecalis*. Madhumathi *et al.* [52] 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 resistant. Katircioglu *et al*. [53] found that ether extract of *Oscillatoria* sp. had antimicrobial activity against *B. subtilis*, *B. cereus*, *B. megaterium*, *E. coli*, *S. aureus*, and *P. aeruginosa*. Ahmadi and Hosseini [54] also revealed that *Oscillatoria* sp. diethyl ether extract showed antibacterial activity against *E. coli* and *B. subtilis*. In contrast, Khairy and El-Kassas [43] reported that *O. angustissina* diethyl ether extract had no antibacterial activity against *B. subtilis*, *B. cereus*, *S. aureus*, *E. coli*, and *P. aeruginosa*. Kim [55] reported that *O. angustissima* ether extract had antifungal activity against *F. oxysporium* and *Alternaria alternata*. Pawar and Puranik [56] indicated that *O. ornata* petroleum ether extract had antifungal activity against *A. niger* and *F. oxysporum*. In contrast, Shanab [38] found that diethyl ether fractions of *O. rubescens*, *O. humelli* and *O. platensis* showed no antifungal activity against *A. flavus*. Also, Padhi *et al*. [57] reported that *O. princeps* ether extract had no antifungal activity against *P. notalum*, *F. moniliforme*, and *A. niger*, while benzene extract had bioactivity against these fungi.

3.4. Minimum inhibitory concentration (MIC) of O. princeps DEE and fractions.

As shown in Fig. 2 and 3, the highest activity of *O. princeps* DEE was recorded against *B. cereus*, *S. aureus*, and *P. aeruginosa* with MIC value of 0.5 mg ml^{-1}. Whereas the lowest activity was showed against *A. ochraceus* with MIC value of 1.8 mg ml^{-1}. *O. princeps* F4 showed the highest activity against *A. westerdijikia* and *A. carbonarus* with MIC 0.8 mg ml^{-1}, and the lowest activity was recorded against *B. cereus* and *P. verrucosum* with MIC 1.9 mg ml^{-1}. The highest activity of F7 with MIC value 0.7 mg ml^{-1} was showed against *P. aeruginosa* and *F. verticelloides*. At the same time, the lowest activity was recorded against *K. pneumonia* and *F. proleferatum* with MIC value of 1.6 mg ml^{-1}.

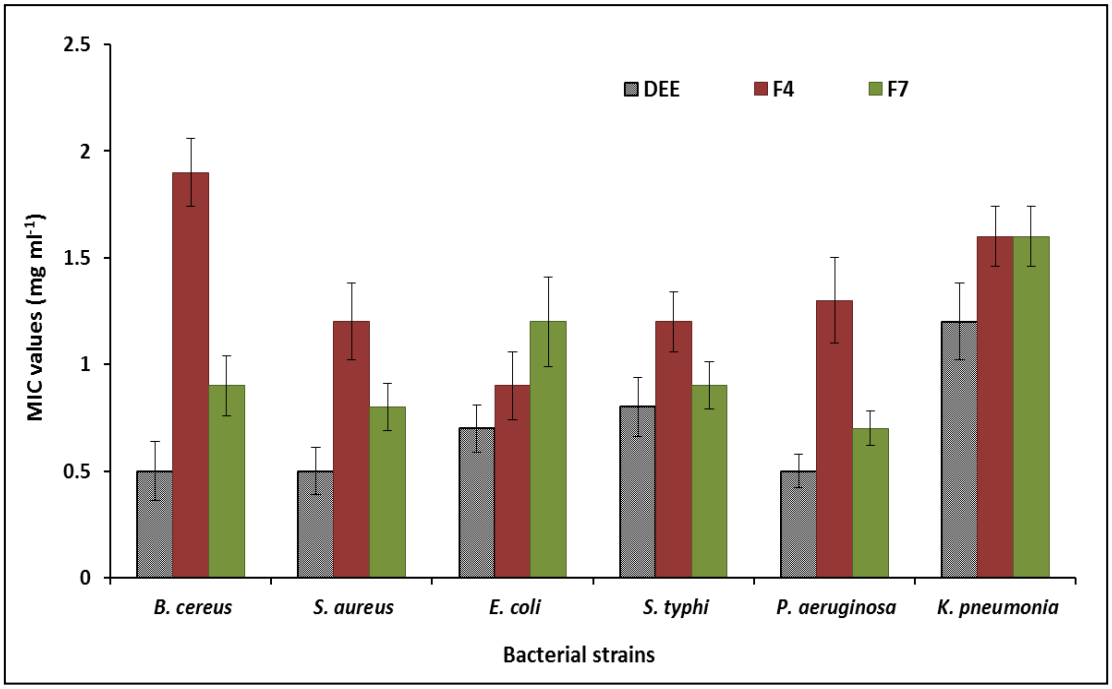

Figure 2. MIC values (mg ml^{-1}) of *O. princeps* DEE and Fractions against pathogenic bacteria.

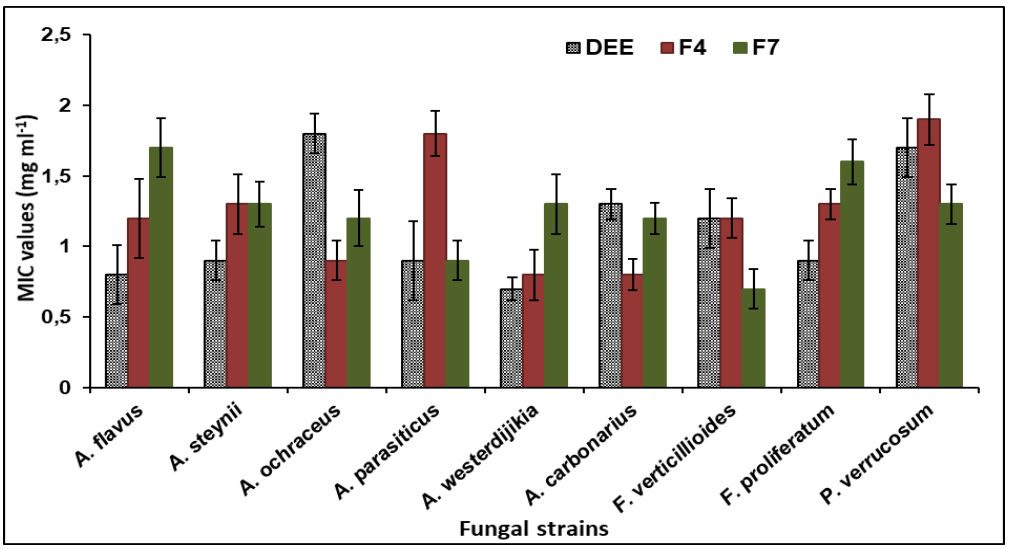

Figure 3. MIC values (mg ml^{-1}) of *O. princeps* DEE and Fractions against toxigenic fungi.

Sethubathi and Prabu [58] reported that aqueous fraction from *Oscillatoria* sp. showed antibacterial activity against *S. aureus*, *B. subtilis*, *P. aeruginosa*, and *K. pneumonia* with MIC values ranged from 0.5 to 2 1.6 mg ml^{-1}. Also, Al-Rekabi [59] found that *O. amoena* aqueous and ethanolic fractions had antibacterial activity against *S. aureus*, *P. aeruginosa*, *B. subtilis*, and *K. pneumonia* with MIC values in a range between 5 to 75 µg ml^{-1}. They also reported that *O. irrigua* aqueous fraction had antifungal activity against *A. flavus* with MIC value of 1.25 mg ml^{-1}.

3.5. Cytotoxic activity of O. princeps DEE fractions.

Since DEE fractions F4 and F7 of *O. princeps* had the highest activity as an antimicrobial agent against tested bacteria and fungi. These fractions were examined for their anticancer activity against HepG2, HCT116, and MCF7 cancer cell lines. The cytotoxicity of *O. princeps* fraction F4 is shown in Fig. 4. The highest anticancer activity was shown against HCT116 cells with IC_{50} of 22.62 µg ml^{-1}, followed by MCF7 cells with IC_{50} of 24.43µg ml^{-1}. While low anticancer activity was observed against HepG2 cell lines at IC_{50} of 102.52 µg ml^{-1}.

The cytotoxicity of *O. princeps* fraction F7 is illustrated in Fig. 5. The highest anticancer activity was recorded against colon cancer cell lines HCT116 with IC_{50} of 30.82 µg ml^{-1}, followed by breast cancer cell lines MCF7 with IC_{50} of 39.73 µg ml^{-1}. While No anticancer activity of *O. princeps* fraction F7 was showed against liver cancer cell lines HepG2 by using concentrations reach 10 mg ml^{-1}.

Roussis *et al*. [60] reported that the lipophilic fractions of *O. acutissima* had shown anticancer activity against colon cancer cell lines HCT116 and breast cancer cell lines MCF7 with IC_{50} of 9.5 and 6.0 µg mg^{-1}, respectively. Shanab *et al*. [47] 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). Also, Nair and Bhimba [49] indicated that the crude extract of *Oscillatoria boryana* possessed anticancer activity against the breast cancer cell lines MCF7. Marrez *et al*. [19] revealed that *Oscillatoria brevis* diethyl ether fraction F4 showed high anticancer activity against MCF7 and HCT116 cell lines with IC_{50} values of 20.6 and 23.4 µg mg^{-1}, respectively.

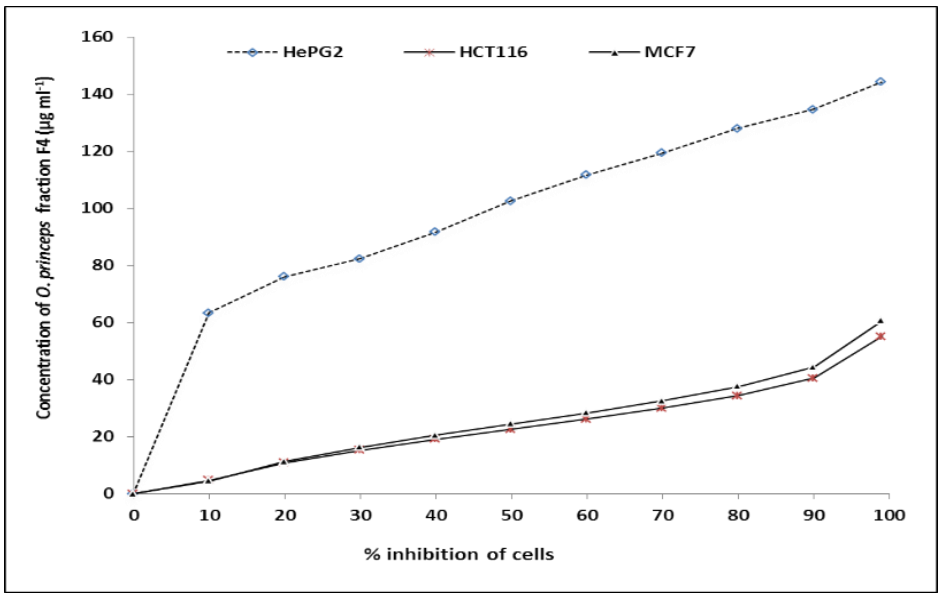

Figure 4. Cytotoxic assay of *O. princeps* diethyl ether extract F4 on HePG2, HCT116, and MCF7 cell lines.

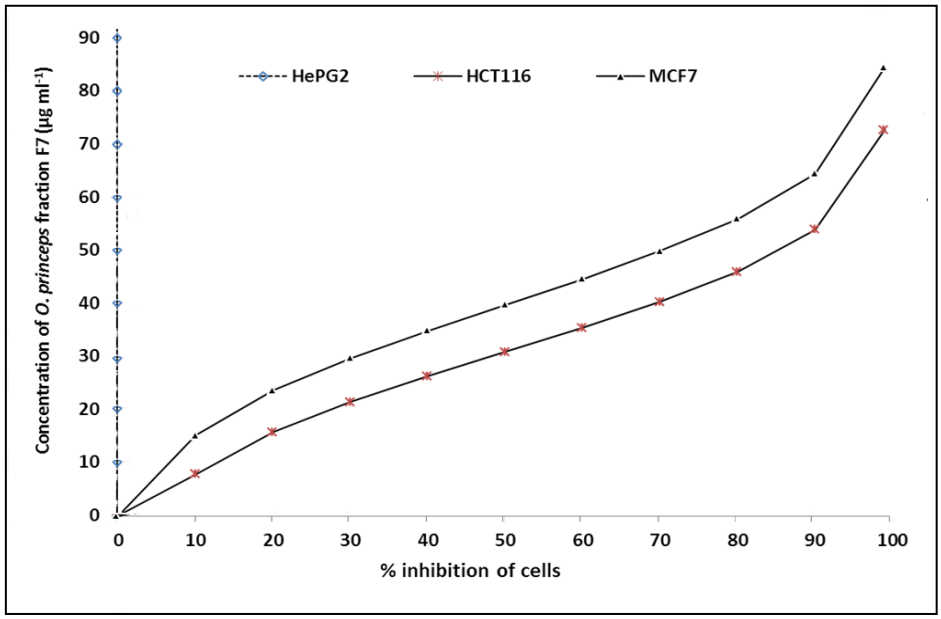

Figure 5. Cytotoxic assay of *O. princeps* diethyl ether extract F7 on HePG2, HCT116, and MCF7 cell lines.

3.6. Identification of O. princeps bioactive fractions using GC-MS.

Totally 10 compounds were identified by GC-MS from *O. princeps* fraction F4. These compounds were 9,12-Octadecadienoic acid (Z,Z)- with peak area percent 8.62%, 9-Octadecenoic acid (15.82%), N-2,4-Dnp-L-arginine (5.34%), Hexadecanoic acid (8.92%), Pentadeconic acid,4 hexadecyl ester (27.26%), 9,15-Octadecadienoic acid, methyl ester (7.66%), 11-Octadecenoic acid, methyl ester (10.65%), Ethyl iso-allocholate (1.77%), Dimethoxyglycerol docosyl ether (11.58) and Stearic acid 4.07% (Fig. 6 and Table 5).

Surendhiran *et al*. [61] reported that the fatty acids Hexadecanoic acid, 9-Octadecenoic acid, and 9,12-Octadecadienoic acid, which isolated from microalgae *Nannochloropsis oculata* showed antibacterial activity against *P. aeruginosa*, *E. coli*, *B. subtilis*, and *S. aureus*. Also, Khalid and Shameel [62] found that fatty acids 9,12-Octadecadienoic acid and 9-Octadecenoic acid from methanol extract of green alga *Spirogyra rhizoides* showed antimicrobial activity against *B. cereus*, *E. coli*, *P. aeruginosa*, *K. pneumonia*, *S. typhi*, *S. faecalis*, *S. pyogenes*, *V. choleriae*, *F. oxysporum*, and *A. flavus*. Marimuthu *et al*. [63] indicated that the fatty acids

Hexadecanoic acid methyl ester, 9-Octadecenoic acid, 9,12-Octadecadienoic acid (Z,Z), 11-Octadecenoic acid methyl ester, and Ethyl iso-allocholate, which extracted from dry Christmas lima bean, had antimicrobial activity against *E. coli*, *S. aureus*, *A. flavus*, and *A. niger*. They also reported that 9-Octadecenoic acid was found to be effective against fungi *A. flavus*. Also, Asghar *et al*. [64] indicated that the bioactive fractions 9-Octadecenoic acid methyl ester and 11-Octadecenoic acid methyl ester from ether extract of Iris plants showed antimicrobial activity against *E. coli*, *B. subtilis*, *S. aureus*, *A. flavus*, and *A. niger*.

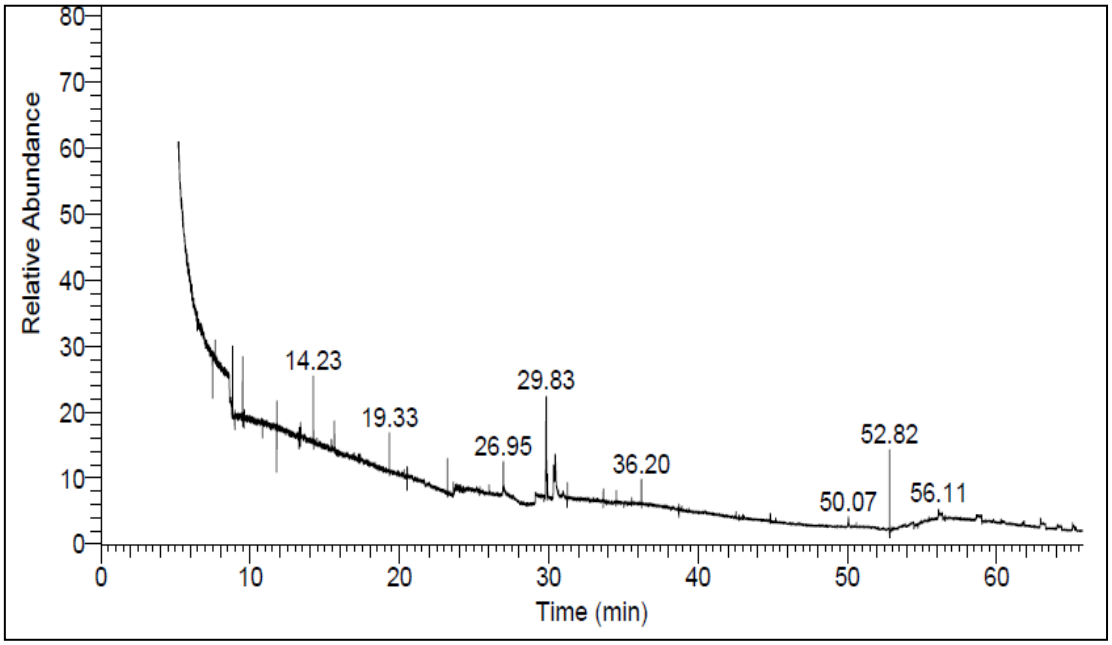

Figure 6. GC-MS chromatogram of *O. princeps* DEE extract fraction F4.

Table 5. Components detected in fraction F4 of *O. princeps* DEE extract.

No	RT	Compound	Area %	Molecular formula	MW
1	10.45	9,12-Octadecadienoic acid (Z,Z)-	8.62	$C_{18}H_{32}O_2$	280
2	14.23	9-Octadecenoic acid	15.82	$C_{18}H_{34}O_2$	282
3	19.33	N-2,4-Dnp-L-arginine	5.34	$C_{12}H_{16}N_6O_6$	340
4	26.95	Hexadecanoic acid	8.92	$C_{16}H_{32}O_2$	256
5	29.83	Pentadeconic acid,4- hexadecyl ester	27.26	$C_{15}H_{30}O_2$	242
6	30.33	9,15-Octadecadienoic acid, methyl ester	7.66	$C_{19}H_{34}O_2$	294
7	36.20	11-Octadecenoic acid, methyl ester	10.65	$C_{19}H_{36}O_2$	296
8	50.07	Ethyl iso-allocholate	1.77	$C_{26}H_{44}O_5$	436
9	52.82	Dimethoxyglycerol docosyl ether	11.58	$C_{27}H_{56}O_5$	460
10	56.11	Stearic acid	4.07	$C_{39}H_{78}O_3$	594

The results about GC-MS analysis of *O. princeps* fraction F7 are illustrated in Fig. 7 and Table 6. It revealed the presence of 5 metabolites with retention time ranging from 17.52 to 63.63 min. The maximum peak was identified as Methyl tetradecanoate (Myristic acid, methyl ester) 64.10%, followed by Quercetin 7,3',4'-trimethoxy 13.48% and Octasiloxane 9.85%. While, the minimum peak was identified as Cis-vaccenic acid 2.42%, followed by 9,12-Octadecadienoic acid (Z,Z) 3.577 and Heptasiloxane 5.94%.

Ahmad *et al*. [65] revealed that 9-Octadecenoic acid and 9,15-Octadecadienoic acid methyl ester from the lipid extract of medicinal plant *Acacia modesta* had antifungal activity against *A. flavus* and *F. solani* as well as cytotoxic activity against *Artemia salina*. While, the lipid fractions were inactive against *E. coli*, *B. subtilis*, *S. aureus*, *P. aeruginosa*, and *S. typhi*. Sheela and Uthayakumari [66] indicated that Ethyl iso-allocholate, 9-Octadecenoic acid, and Hexadecanoic acid from ethanolic extract of costal plant *Sesuvium portulacastrum* had

antimicrobial, anticancer, and antioxidant activities. Rajalakashmi and Mahesh [67] reported that bioactive metabolites Octasiloxane, 9,12-Octadecadienoic acid (Z,Z) and Heptasiloxane, which extracted from *A. terrus* in rhizosphere soil of medicinal plants, had antibacterial activity against *K. pneumonia*, *E. coli*, *B. subtilis*, *S. aureus*, and *P. aeruginosa*. Salem *et al*. [68] reported that Quercetin 7,3',4'-trimethoxy from *Nostoc* sp. methanol extract had antimicrobial activity against *B. subtilis*, *K. pneumonia*e, *S. aureus*, and *A. niger*. Also, Marrez *et al*. [8] found that 9-Octadecenoic acid, Quercetin 7,3',4'-trimethoxy, and Octasiloxane isolated from *S. obliquus* diethyl ether fractions had antibacterial activity against *B. cereus*, *Staph. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, *K. pneumonia* and antifungal activity against *A. flavus*, *A. steynii*, *A. ochraceus*, *A. parasiticus*, *A. westerdijikia*, *A. carbonarius*, *F. verticillioides*, *F. proliferatum*, and *P. verrucosum*.

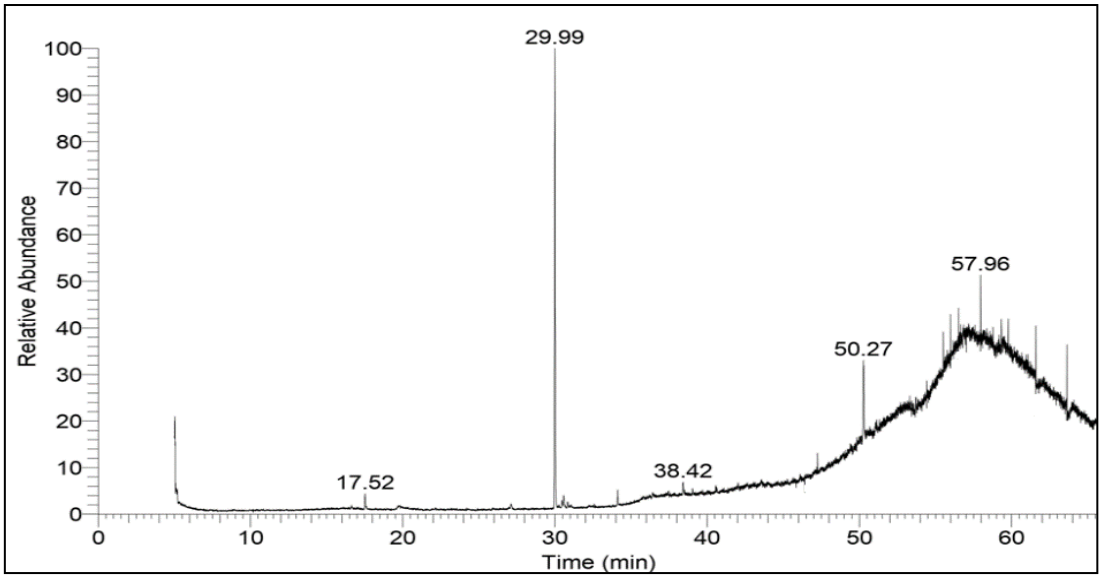

Figure 7. GC-MS chromatogram of *O. princeps* DEE extract fraction F7.

Table 6. Components detected in fraction F7 of *O. princeps* DEE extract.

No	RT	Compound	Area %	Molecular formula	MW
1	17.52	9,12-Octadecadienoic acid (Z,Z)-	3.57	$C_{18}H_{32}O_2$	280
2	29.99	Methyl tetradecanoate (Myristic acid, methyl ester)	64.10	$C_{15}H_{30}O_2$	242
3	30.57	Cis-vaccenic acid	2.42	$C_{18}H_{34}O_2$	282
4	50.27	Quercetin 7,3',4'-trimethoxy	13.48	$C_{18}H_{16}O_7$	344
5	57.96	Octasiloxane	9.85	$C_{16}H_{50}O_7Si_8$	578
6	63.63	Heptasiloxane	5.94	$C_{14}H_{44}O_6Si_7$	504

Udgire and Pathade [69] reported that Pentadeconic acid, 4- hexadecyl ester, isolated from the medicinal plant *Valeriana wallichii* showed had antimicrobial activity against *K. pneumonia*, *E. coli*, *S. aureus*, *P. aeruginosa*, and *A. niger*. Hamlal and Subban [70] found that Cis-vaccenic acid, 9,12-Octadecadienoic acid, and 11-Octadecenoic acid, methyl ester from methanolic extract of *P. viscida* and *D. gangeticum* dried roots possessed antimicrobial activity against *B. cereus*, *S. aureus*, *A. flavus*, *A. terrus*, and *P. notatum*. Laungsuwon and Chulalaksananukul [71] indicated that Methyl tetradecanoate (Myristic acid, methyl ester) extracted from microalgae *C. glomerata* and *M. floccosa* showed antibacterial activity against *B. cereus* and *S. aureus*. Marrez and Sultan [72] revealed that 9,12-Octadecadienoic acid and Pentadeconic acid,4 hexadecyl ester which isolated from *Microcystis aeruginosa* diethyl ether sub-fraction F4-10 had antifungal activity against *A. flavus*, *A. niger*, *A. ochraceus*, *A. parasiticus*, *A. westerdijikia*, *A. carbonarius*, *F. proliferatum*, *F. verticillioides*, and *P. verrucosum*. Abdel-Rahman *et al*. [73] found that 9-Octadecenoic acid, Octasiloxane, and 9,12-

Octadecadienoic acid from *Microcystis aeruginosa* diethyl ether fraction F7 showed antibacterial activity against *B. cereus*, *S. aureus*, *E. coli*, *P. aeruginosa*, *S. typhi*, *K. pneumonia*, and anticancer activity HCT116 cell line.

4. Conclusions

The current study demonstrated that *O. princeps* a rich source of bioactive compounds with antimicrobial activity wide range of foodborne pathogenic bacteria and mycotoxigenic fungi. Furthermore, these extracts exhibited anticancer activity against HePG2, HTC116, and MCF7 cell lines. Also, *O. princeps* DEE fraction showed bioactivity against pathogenic microorganisms and cancer cell lines which qualifies it to be a safe and cheap source for pharmaceutical and food bio-preservative ingredients.

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Conflicts of Interest

The authors declare no conflict of interest.

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