Heliyon

Received:
21 September 2018
Revised:
2 February 2019
Accepted:
19 March 2019

Cite as: Diaa A. Marrez, Mohamed M. Naguib, Yousef Y. Sultan, Aziz M. Higazy. Antimicrobial and anticancer activities of *Scenedesmus obliquus* metabolites. Heliyon 5 (2019) e01404. doi: 10.1016/j.heliyon.2019.e01404

Antimicrobial and anticancer activities of *Scenedesmus obliquus* metabolites

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Abstract

This study was conducted to evaluate the antimicrobial activities and cytotoxicity of both crude extracts of *Scenedesmus obliquus* and their fractions. *In vitro* cytotoxicity assay against human hepatocellular carcinoma (HepG2), colon cancer (HCT116) and breast cancer (MCF7) cell line was monitored. The highest inhibition was observed using diethyl ether crude extract (DEE) recording between 12.5 and 19.5 mm inhibition zone against all tested bacteria and between 8.7 and 18.3 mm against tested fungi. The highest anticancer effect of DEE was observed at IC_{50} against HCT116 and HepG2 cell lines using just 24.6 and 42.8 μg ml^{-1}, respectively. While, high concentration, 93.8 μg ml^{-1}, was required to exhibit its effect against MCF7. Column chromatography technique was used to separate DEE crude extract to its main components using 7 different mobile phases. Fractions F1 and F7 were the highest fractions that had antimicrobial activity against tested bacteria and fungi. High $IC_{50} > 80$ μg ml^{-1} were required to exhibit anticancer activity at IC_{50} against the tested cancer cell lines. The main compounds responsible for the bioactivity were identified using GC-MS, nonadecane and butylated hydroxytoluene in F1 and 9-octadecadienoic acid and quercetin 7,3′,4′-trimethoxy in F7 were identified. The current study highlights the potential use of *S. Obliquus* extract and their fractions as a source of antimicrobial and anticancer compounds.

Keywords: Cancer research, Microbiology, Natural product chemistry

https://doi.org/10.1016/j.heliyon.2019.e01404

1. Introduction

Microalgae are a diverse group of prokaryotic and eukaryotic photosynthetic microscopic algae 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. Microalgae are found in a wide range of different habitats from fresh to marine and hyper-saline environments [1]. In general, microalgae are rich in various valuable compounds like carotenoids, phycocyanin, phenolics, amino acids, polyunsaturated fatty acids, and sulphated polysaccharides that are used as feedstock for biodiesel production, animal feed, food additives, cosmetics and medicine [2, 3]. These compounds are providing excellent various biological activities including, antioxidant, antimicrobial, antiviral, antitumoral, anti-inflammatory and anti-allergy effects [4, 5].

Some microalgae species such as *Chlorella* spp., *Spirulina* spp., *Dunaliella* spp. and *Scenedesmus* spp. have recently drawn attention as commercially valuable sources of a wide spectrum of bioactive compounds [6]. *Scenedesmus* is one of the most common freshwater algae genera. Due to the easy of *Scenedesmus* culturing, harvesting and drying process, it becomes the most common popular species in microalgal biotechnology studies [7]. *Scenedesmus* sp. appears to be a rich source of new antimicrobial and anticancer compounds. Several studies reported the antimicrobial bacterial activity of *Scenedesmus obliquus* and *S. quandricanda* against different species of foodborne pathogenic bacteria and mycotoxigenic fungi [8, 9]. Also, *S. obliquus* extracts had anticancer activity against human breast MCF7, hepatic HePG2, Colon HCT116 and human cervical adenocarcinoma HeLa cancer cell line [10, 11].

Objectives of this study were to evaluate *S. obliquus* extract and its fractions antibacterial activity against pathogenic bacteria, antifungal activity against mycotoxigenic fungi and anticancer activity against liver, colon and breast cancer cell lines. Also, identify the chemical profile of the active fractions against various microbes and cancer cell lines using GC-MS Technique.

2. Materials and methods

2.1. Algal strain and culture medium

Pure isolate of *Scenedesmus obliquus* was obtained from Marine Toxins Lab., National Research Centre, Egypt [12]. The culture medium used for cultivation was BG-11 [13]. At the stationary phase of growth, 25 days, *S. obliquus* biomass was harvested and dried overnight in a hot air oven at 50 °C.

https://doi.org/10.1016/j.heliyon.2019.e01404

2.2. Preparation of microalgae extracts

The dried *S. obliquus* biomass (20 g) was homogenized separately in water and different organic solvents such as methanol, ethanol, acetone, chloroform, diethyl ether, ethyl acetate and hexane (HPLC grade, Sigma-Aldrich). Each homogenized biomass was sonicated for 20 min using ultrasonic micro tip probe of 400 watt (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 40°C using rotary evaporator. Dried extracts were kept in labeled sterile vials in a deep freezer at −20 °C till further use [14].

2.3. Antimicrobial activity

2.3.1. Test microorganisms

The antimicrobial activity of *S. obliquus* crude extracts and fractions 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.

2.3.2. Disc diffusion method

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 achieves the turbidity of 0.5 McFarland $BaSO_4$ standard. The bioactivity of *S. obliquus* crude extracts and its fractions were examined against all the tested bacterial species using disc diffusion method of Kirby-Bauer technique [15, 16]. 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. 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

https://doi.org/10.1016/j.heliyon.2019.e01404

then the inhibition zones were measured and expressed as the diameter of clear zone including the diameter of the paper disc.

The fungal strains were plated onto potato dextrose agar (PDA) and incubated for 5 days at 25 °C. The 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 yeast extract sucrose medium (YES) 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. DMSO and commercial fungicide Nystatin (1000 Unit ml^{-1}) were considered as a negative and positive control, respectively. The inoculated plates were incubated at 25°C for 48 h, then the antifungal activity was assessed by measuring the zone of inhibition (mm) [15]. The results average was calculated from at least three replicates for each assay.

2.3.3. Determination of minimum inhibitory concentration (MIC)

The determination of MIC was conducted using tube dilution method [17]. A 24 h culture of the tested bacterial species was diluted in 10 ml of tryptic soy broth (TSB) with reference to the 0.5 McFarland standard to achieve inocula of 10^8 cfu ml^{-1}. In culture tube containing nine different concentrations of each *S. obliquu*s 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 μl of bacterial cell suspension and incubated at 37 °C for 24 h. The growth of the inoculum in broth is indicated by turbidity of the broth and the lowest concentration of the extract which inhibited the growth of the test organism was taken as the minimum inhibitory concentration (MIC).

MIC against fungi was performed by using the technique of Perrucci *et al.* [18]. Crude extracts and fractions at different concentrations were separately dissolved in 0.5 ml of 0.1% Tween 80 (Merck, Darmstadt, Germany), then mixed with 9.5 ml of melting, 45 °C, PDA and poured into Petri dish (6 cm). The prepared plates were centrally inoculated with 3 μl of fungal suspension (10^8 cfu ml^{-1}; 0.5 McFarland standard). The plates were incubated at 25 °C for 24−48 h. At the end of the incubation period, mycelial growth was monitored and MIC was determined.

2.4. *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 [19]. Three human cancer cell lines, hepatocellular carcinoma (HepG2), colon cancer (HCT116) and breast cancer (MCF7) were subjected to *S. obliquu*s

https://doi.org/10.1016/j.heliyon.2019.e01404

extracts and its fractions. Cells were suspended in RPMI 1640 medium in 96-well microtiter plastic plates at concentration of 10×10^3 cells/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 four hours 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 Novantron standard (100 μg ml^{-1}) was used as a known cytotoxic natural agent who gives 100% lethality under the same conditions [20].

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) $\times$ 100. A probit analysis was carried for IC_{50} and IC_{90} determination using SPSS 11 program.

2.5. Fractionations of *S. obliquus* DEE crude extract

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 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 [3].

2.6. GC/MS analysis

The diethyl ether fraction F1 and F7 were subjected to analysis of chemical composition by using GC/MS, Thermo Scientific, Trace GC Ultra coupled with ISQ Single

https://doi.org/10.1016/j.heliyon.2019.e01404

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 to 150 °C at 7 °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 [3]. 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.

The methodology of the study is summarized and illustrated in Fig. 1.

2.7. Statistical analysis

Statistical significance was determined using Statistica Version 9 (StateSoft, 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.

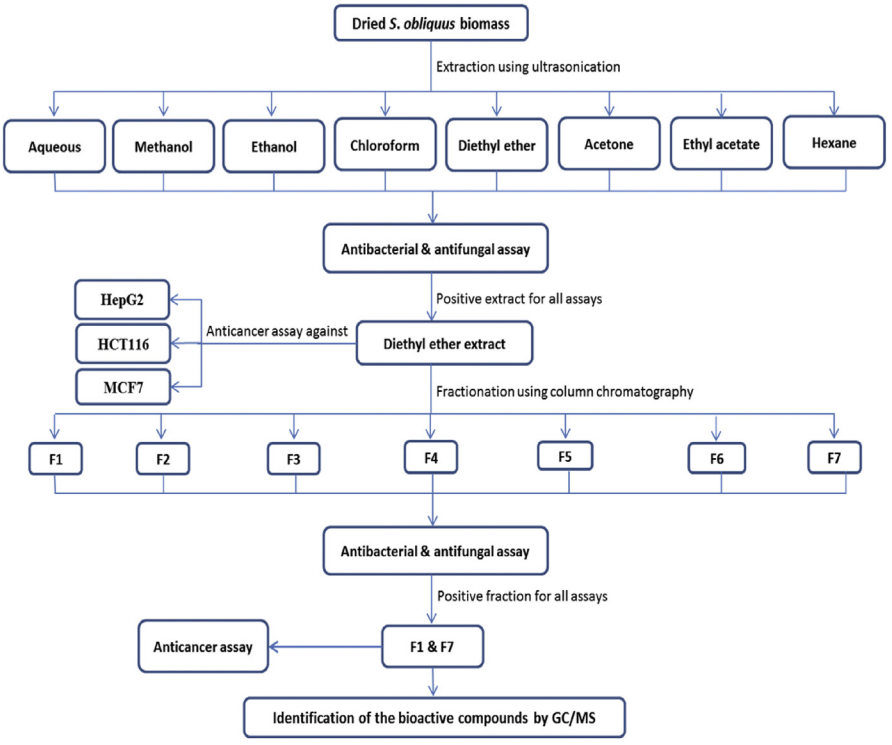

Fig. 1. Flow chart of extraction, fractionation and identification of bioactive compounds from *S. obliquus*.

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3. Results and discussion

3.1. Antimicrobial activity of *S. obliquus* crude extracts

As shown in Table 1 the antibacterial activity of *S. obliquus* extracts against 6 strains of pathogenic bacteria. Each of aqueous, chloroform and diethyl ether extracts had antibacterial activity against all tested pathogenic bacteria followed by ethyl acetate extract which showed antibacterial activity against all tested bacteria except *P. aeruginosa*. The diethyl ether extract showed the highest antibacterial activity against *S. typhi* and *P. aeruginosa* which recorded 19.5 and 18.5 mm inhibition zones, respectively. The acetone extract had not antibacterial activity against all tested bacteria.

The antibacterial activity of *S. obliquus* extracts (Table 1) is in accordance with Tuney *et al.* [21] study which reported that *Scenedesmus* sp. methanol, acetone and ethanol extracts had moderate antibacterial activity against *P. aeruginosa* and *E. coli*, while diethyl ether extract gave the highest results against these bacteria. Abedin and Taha [8] found that *Scenedesmus quandricanda* methanolic extract showed antibacterial activity against *E. coli*, *B. cereus* and *S. aureus*, while ethanol, acetone and diethyl ether extracts had antibacterial activity against *B. cereus* and *S. aureus*. Also, they found that *P. aeruginosa* showed resistant to ethanolic, acetone, diethyl ether and methanol extracts of *S. quandricanda*. Nair and Krishnika [22] reported that *Scenedesmus* sp. acetone, methanol, diethyl ether and hexane extracts had moderate antibacterial activity against *Pseudomonas* sp., while aqueous showed remarkable antibacterial property as compared to other extracts. In contrast, Najdenski *et al.* [9] reported that *Scenedesmus obliquus* ethanolic extract had antibacterial activity against *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa* and *S. typhi*.

Table 2 illustrates the antifungal activity of *S. obliquus* extracts against different species of mycotoxigenic fungi. The highest antifungal activity of *S. obliquus* extracts was shown against *A. steynii* followed by *A. carbonarius* with diethyl ether extract which had 18.3 and 15.7 mm inhibition zones, respectively. Diethyl ether extract had antifungal activity against all tested mycotoxigenic fungi followed by aqueous, methanol, chloroform, ethyl acetate ad hexane extracts which showed antifungal activity against four fungal species from the nine tested fungi. *A. ochraceus*, *F. verticillioides* and *P. verrucosum* showed resistance against all *S. obliquus* extracts with exception of diethyl ether extracts which had 9.3, 13.7 and 8.7 mm inhibition zone, respectively.

In contrast with the current study Ghasemi *et al.* [23] found that *S. obliquus* culture filtrate, aqueous, methanol and hexane extracts had antifungal activity against *A. fumigatus* and *A. niger*. Abedin and Taha [8] reported that *Scenedesmus quandricanda* ethanol, acetone, diethyl ether and methanolic extracts have antifungal activity against *A. niger*, *A. flavus*, *P. herquei* and *F. moilifore*. The highest inhibition zone was recorded in diethyl ether extract, this result is in agreement with the present

https://doi.org/10.1016/j.heliyon.2019.e01404

Table 1. Antibacterial activity of *Scenedesmus obliquus* crude extracts.

Bacteria	Inhibition zone mm (Mean ± *S.E)									
	−ve control	+ve control	Aqueous	MeOH	EtOH	Acetone	CH_3Cl	DEE	EtOA	Hexane
B. cereus	–	16.7 ± 1.15^{a}	9.2 ± 0.76^{c}	9.0 ± 1.32^{c}	8.5 ± 0.50^{d}	–	8.8 ± 0.29^{cd}	14.7 ± 2.25^{b}	8.3 ± 0.76^{d}	8.3 ± 1.04^{d}
S. aureus	–	17.3 ± 0.76^{a}	9.5 ± 0.50^{c}	9.7 ± 0.76^{c}	9.7 ± 1.04^{c}	–	9.5 ± 0.50^{c}	12.5 ± 2.00^{b}	8.5 ± 0.50^{d}	8.3 ± 1.15^{d}
E. coli	–	18.8 ± 1.61^{a}	10.2 ± 1.25d^{e}	9.7 ± 1.04^{e}	11.8 ± 2.36^{c}	–	9.5 ± 1.80^{f}	17.2 ± 2.46^{b}	11.3 ± 2.47^{d}	9.7 ± 1.52^{e}
S. typhi	–	15.5 ± 0.50^{c}	10.2 ± 0.76^{ef}	9.5 ± 0.5^{f}	17.0 ± 0.50^{b}	–	11.3 ± 1.25^{e}	19.5 ± 0.5^{a}	9.3 ± 1.44^{f}	12.8 ± 1.56^{d}
P. aeruginosa	–	19.5 ± 0.50^{a}	15.3 ± 0.28^{c}	–	–	–	9.7 ± 1.61^{d}	18.5 ± 2.18^{b}	–	–
K. pneumoniae	–	18.5 ± 2.50^{a}	7.8 ± 0.76^{d}	–	–	–	8.5 ± 0.50^{c}	12.7 ± 1.61^{b}	7.7 ± 0.76^{d}	–

n = 3, *S.E: standard error, different subscripts within row 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.

https://doi.org/10.1016/j.heliyon.2019.e01404

Table 2. Antifungal activity of *Scenedesmus obliquus* crude extracts.

Fungi	Inhibition zone mm (Mean ± *S.E)									
	−ve control	+ve control	Aqueous	MeOH	EtOH	Acetone	CH_3Cl	DEE	EtOA	Hexane
A. flavus	–	16.1 ± 0.74^{a}	11.5 ± 1.59^{c}	9.7 ± 0.76^{d}	8.5 ± 0.86^{e}	–	8.3 ± 0.58^{e}	13.3 ± 1.04^{b}	13.3 ± 1.75^{b}	8.2 ± 0.76^{e}
A. steynii	–	7.5 ± 0.35^{d}	7.5 ± 0.86^{d}	–	–	–	7.3 ± 0.28^{d}	18.3 ± 2.36^{a}	9.2 ± 1.25^{b}	8.0 ± 0.86^{c}
A. ochraceus	–	10.9 ± 0.54^{a}	–	–	–	–	–	9.3 ± 1.61^{b}	–	–
A. parasiticus	–	11.8 ± 2.01^{b}	–	10.7 ± 0.76^{c}	–	7.7 ± 0.76^{d}	7.8 ± 1.04^{d}	14.3 ± 2.02^{a}	7.8 ± 0.76^{d}	–
A. westerdijikia	–	10.5 ± 0.35^{a}	8.3 ± 0.58^{b}	8.5 ± 0.86^{b}	7.2 ± 0.28^{c}	–	–	10.5 ± 1.32^{a}	7.5 ± 0.86c	–
A. carbonarius	–	10.4 ± 0.41^{b}	7.7 ± 0.58^{c}	–	–	–	7.3 ± 0.28^{c}	15.7 ± 1.25^{a}	–	7.3 ± 0.58^{c}
F. verticillioides	–	11.0 ± 0.35^{b}	–	–	–	–	–	13.7 ± 2.40^{a}	–	–
F. proliferatum	–	11.0 ± 0.61^{a}	–	7.8 ± 0.28^{d}	7.8 ± 0.28^{d}	7.5 ± 0.50^{d}	–	10.0 ± 1.32^{b}	–	9.7 ± 1.15^{c}
P. verrucosum	–	9.9 ± 1.43^{a}	–	–	–	–	–	8.7 ± 0.58^{b}	–	–

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

https://doi.org/10.1016/j.heliyon.2019.e01404

result. Also, Al-Rekabi [24] found that *Scenedesmus quandricanda* aqueous extract had antifungal activity against *A. flavus.*

3.2. Antimicrobial activity of *S. obliquus* DEE fractions

Table 3 illustrates the antibacterial activity of *S. obliquus* diethyl ether extract fractions. Fraction F7 had antibacterial activity against all tested bacteria followed by F1 which had antibacterial activity against all tested bacteria except *P. aeruginosa*. In contrast, F5 only inhibited *E. coli*. *P. aeruginosa* showed resistance against all fractions except F7, whereas *E. coli* was susceptible to all fractions. The highest antibacterial activity was recorded by F7 against *S. typhi* with inhibition zone of 9.7 mm.

The antifungal activity of *S. obliquus* DEE fractions against nine mycotoxigenic fungi are shown in Table 4. F7 was the best fraction since it had antifungal activity against tested fungi except *A. carbonarus*. Following to F7, Fraction F1 had antifungal activity against tested fungi except *A. flavus* and *A. westerdijikia*. In contrast, F5 had no activity against all tested fungi followed by F3 which had bioactivity only against *A. stenyii*. Both of *A. flavus* and *A. carbonarus* showed resistance against all fractions except F7 and F1, respectively. The highest inhibition zone, 11.3 mm, was recorded using F6 against *A. stenyii* followed by 11.0 mm using F1 against *P. verrucosum*.

Najdenski *et al.* [9] found that phenolic extract of *S. obliquus* had antibacterial activity against *S. aureus* and *B. cereus*. Desbois *et al.* [25] indicated that the partially purified fatty acids from *Scenedesmus costatum* exhibited strong activity against human pathogens as *E. coli*, *P. aeruginosa* and *S. aureus*. Aremu *et al.* [26] reported that ether extracts of *Scenedesmus* sp. and *S. quadricauda* at different culture age possessed antibacterial activity against *S. aureus*, *E. coli* and *P. aeruginosa*. Nair and Krishnika [22] tested the antimicrobial activity of *Scenedesmus* sp. isolated

Table 3. Antibacterial activity of *Scenedesmus obliquus* DEE extracts fractions.

Bacteria	Inhibition zone mm (Mean ± S.E)							
	−ve control	+ve control	F1	F2	F3	F4	F5	F6	F7
B. cereus	–	16.7 ± 1.15^{a}	7.3 ± 0.58^{c}	–	–	8.3 ± 1.15^{b}	–	–	7.0 ± 0.28^{c}
S. aureus	–	17.3 ± 0.76^{a}	8.3 ± 1.52^{b}	–	–	–	–	–	7.7 ± 0.58^{c}
E. coli	–	18.8 ± 1.61^{a}	9.0 ± 0.15^{b}	8.3 ± 1.15^{c}	8.3 ± 1.19^{c}	7.0 ± 0.28^{d}	7.0 ± 0.15^{d}	9.3 ± 0.58^{b}	9.0 ± 0.28^{b}
S. typhi	–	15.5 ± 0.50^{a}	8.3 ± 1.15^{c}	–	–	8.7 ± 0.58^{c}	–	–	9.7 ± 0.58^{b}
P. aeruginosa	–	19.5 ± 0.50^{a}	–	–	–	–	–	–	8.0 ± 1.0^{b}
K. pneumoniae	–	18.5 ± 2.50^{a}	7.7 ± 0.58^{d}	8.0 ± 1.00^{c}	8.0 ± 1.00^{c}	7.7 ± 0.58^{d}	–	8.7 ± 0.58^{b}	8.0 ± 0.28^{c}

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

https://doi.org/10.1016/j.heliyon.2019.e01404

Table 4. Antifungal activity of *Scenedesmus obliquus* DEE extract fractions.

Fungi	Inhibition zone mm (Mean ± S.E)							
	−ve control	+ve control	F1	F2	F3	F4	F5	F6	F7
A. flavus	–	16.1 ± 0.74^{a}	–	–	–	–	–	–	7.5 ± 0.32^{b}
A. steynii	–	7.5 ± 0.35^{e}	10.3 ± 1.52^{b}	8.5 ± 1.29^{d}	8.0 ± 0.73^{d}	9.0 ± 1.00^{c}	–	11.3 ± 2.08^{a}	9.0 ± 1.00^{c}
A. ochraceus	–	10.9 ± 0.54^{a}	7.7 ± 1.15^{c}	–	–	8.0 ± 1.00^{b}	–	–	8.0 ± 1.00^{b}
A. parasiticus	–	11.8 ± 2.01^{a}	8.7 ± 1.52^{c}	–	–	7.7 ± 1.15^{d}	–	–	9.3 ± 0.058^{b}
A. westerdijikia	–	10.5 ± 0.35^{a}	–	–	–	8.0 ± 1.00^{b}	–	–	8.3 ± 1.15^{b}
A. carbonarius	–	10.4 ± 0.41^{a}	9.7 ± 0.58^{b}	–	–	–	–	–	–
F. verticillioides	–	11.0 ± 0.35^{a}	7.7 ± 0.58^{c}	–	–	–	–	7.3 ± 0.58^{c}	8.3 ± 1.52^{b}
F. proliferatum	–	11.0 ± 0.61^{a}	9.0 ± 0.15^{b}	9.0 ± 0.00^{b}	–	–	–	–	8.3 ± 1.15^{c}
P. verrucosum	–	9.9 ± 1.43^{b}	11.0 ± 2.00^{a}	–	–	7.7 ± 1.15^{d}	–	–	9.3 ± 0.58^{c}

n = 3, *S.E: standard error, different subscripts within row are significantly different at the 5% level, –: No inhibition, negative control: DMSO, positive control: Nystatin.

from natural ponds against three pathogenic bacteria with different solvents; they found that methanolic fractions had better results.

Salem *et al.* [27] reported that *Scenedesmus* sp. methanolic fraction had antibacterial activity against *B. subtilis* and *S. aureus* as well as methanol and acetone fractions had antifungal activity against *F. oxysporum*. Also, Mudimu *et al.* [28] reported that *S. obliquus* methanolic fractions had antimicrobial activity against *E. coli*, *B. subtilis*, *B. cereus*, *P. aeruginosa*, *C. albicans* and *S. cerevisiae*. Abbassy *et al.* [29] revealed that lipid and phenolic extract of *Scenedesmus* sp. had antifungal activity against *F. oxysporum*, *A. niger* and *P. digitatum*.

3.3. Minimum inhibitory concentration (MIC) values of *S. obliquus* DEE and fractions

Table 5 illustrates minimum inhibitory concentration of *S. obliquus* diethyl ether crude extract and fractions F1 and F7. The highest activity of *S. obliquus* DEE was recorded against *S. typhi* and *A. steyni*i with MIC values 0.5 and 0.6 mg ml^{-1}, respectively. Whereas, the lowest activity was showed against *A. ochraceus* and *A. carbonarus* with MIC value 1.8 mg ml^{-1}. *S. obliquus* fraction F1 showed highest activity against *P. verrucosum* and *E. coli* with MIC values 0.7 and 0.8 mg ml^{-1}, respectively, while the lowest activity was recorded against *P. aeruginosa* and *A. flavus* with MIC value 1.9 mg ml^{-1}. The highest activity of F7 with MIC value 0.8 mg ml^{-1} was recorded against *S. typhi* and *A. steynii*. Whereas, the lowest activity was recorded against *A. carbonarus* with MIC value 1.9 mg ml^{-1}.

Desbois *et al.* [25] revealed that hexane extract and partially purified fractions of *Scenedesmus costatum* had antibacterial activity against *E. coli*, *P. aeruginosa*,

https://doi.org/10.1016/j.heliyon.2019.e01404

Table 5. MIC values (mg ml^{-1}) of *S. obliquus* DEE and fractions.

Microorganisms	MIC values mg ml^{-1} (Mean ± S.E)		
Bacteria	**DEE***	**F1**	**F7**
B. cereus	0.8 ± 0.14[a]	1.6 ± 0.32[b]	1.7 ± 0.28[b]
S. aureus	1.2 ± 0.21[a]	1.2 ± 0.28[a]	1.3 ± 0.21[a]
E. coli	0.7 ± 0.11[a]	0.8 ± 0.21a[b]	0.9 ± 0.11[b]
S. typhi	0.5 ± 0.14[a]	1.2 ± 0.28[c]	0.8 ± 0.14[b]
P. aeruginosa	0.7 ± 0.14[a]	1.9 ± 0.32[c]	1.3 ± 0.28[b]
K. pneumoniae	1.2 ± 0.28[a]	1.6 ± 0.14[b]	1.2 ± 0.11[a]
Fungi	**DEE***	**F1**	**F7**
A. flavus	1.2 ± 0.28[a]	1.9 ± 0.32[b]	1.3 ± 0.28[a]
A. steynii	0.6 ± 0.14[a]	1.7 ± 0.28[c]	0.8 ± 0.14[b]
A. ochraceus	1.8 ± 0.28[b]	1.3 ± 0.21[a]	1.2 ± 0.28[a]
A. parasiticus	0.9 ± 0.14[a]	1.8 ± 0.28[b]	0.9 ± 0.11[a]
A. westerdijikia	1.2 ± 0.21[a]	1.2 ± 0.28[a]	1.2 ± 0.25[a]
A. carbonarius	1.8 ± 0.11[b]	0.8 ± 0.11[a]	1.9 ± 0.14[b]
F. verticillioides	0.9 ± 0.11[a]	1.6 ± 0.14[c]	1.2 ± 0.28[b]
F. proliferatum	1.2 ± 0.28[b]	0.9 ± 0.11[a]	1.3 ± 0.28[c]
P. verrucosum	1.3 ± 0.21[c]	0.7 ± 0.08[a]	0.9 ± 0.14[b]

n = 3, *S.E: standard error, different subscripts within row are significantly different at the 5% level, DEE*: Diethyl ether extract.

S. typhi, *S. aureus* and *S. epidermidis* with MIC values ranged between 1.9 and 7.8 μg ml^{-1}. Najdenski *et al.* [3] reported that *Scenedesmus obliquus* ethanolic fractions had antibacterial activity against *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa* and *S. typhi* with MIC values ranged from 0.3 to 3 mg ml^{-1}. Al-Rekabi [24] found that polar fractions of *S. quadricanda* had antifungal activity against *A. flavus* with MIC value 1 mg ml^{-1} Ördög *et al.* [30] reported that *Scenedesmus* sp. lipid extract and partially purified fractions showed antimicrobial activity against several pathogenic bacteria, filamentous fungi and yeasts with MIC values $>$ 0.2 mg ml^{-1}.

3.4. Cytotoxic activity of DEE *S. obliquus* crude extracts

The cytotoxicity of *S. obliquus* diethyl ether extract against HePG2, HCT116 and MCF7 cell lines are illustrated in Fig. 2. Small amounts, 24.62 and 42.77 μg ml^{-1} were required to inhibit 50% (IC_{50}) of HCT116 and HepG2 cell lines, respectively. Whereas, higher concentration 93.77 μg ml^{-1} was used to does that effect on MCF7 cell lines. The cytotoxicity of negative control (DMSO) was less than 1% inhibition at 100 ppm. Also, ether extract showed no effect on proliferation of normal human

https://doi.org/10.1016/j.heliyon.2019.e01404

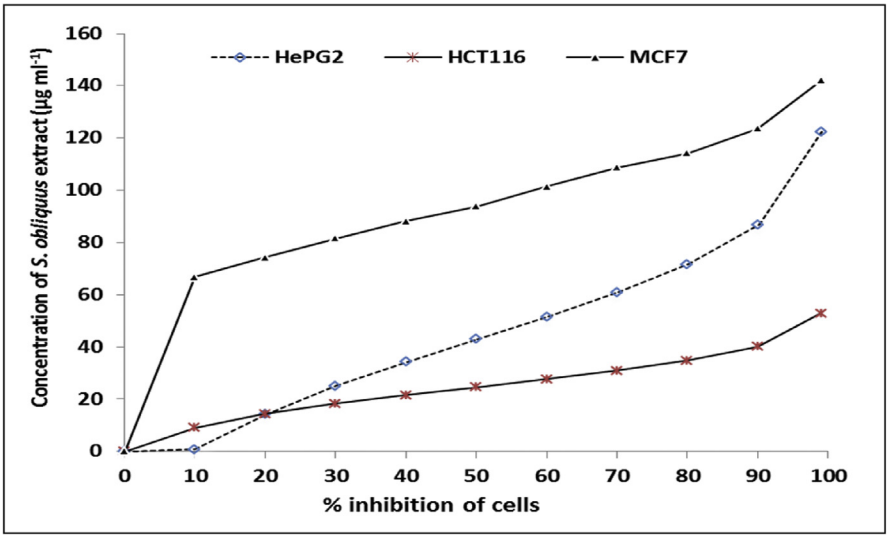

Fig. 2. Cytotoxic assay of *S. obliquus* diethyl ether extract on HepG2, HCT116 and MCF7 cell lines.

epithelial amnion cells WISH and normal fibroblast cell line BJ1. Abd El Baky *et al.* [10] reported that *S. obliquus* oil which extracted with chloroform: methanol (2:1 v/v) showed high anticancer activity against human breast MCF7, hepatic HepG2 and colon HCT116 cancer cell lines at IC_{50} values 11.62, 14.5 and 15.22 µg ml^{-1}, respectively. Also, Silambarasan *et al.* [11] found that *S. obliquus* methanolic extract showed high cytotoxic activity against human cervical adenocarcinoma HeLa cell line at IC_{50} of 50 µg ml^{-1}. Custodio *et al.* [31] studied the effect of *Scenedesmus* sp. hexane, diethyl ether, acetone and aqueous extracts on hepatocellular carcinoma HepG2 cell lines viability. They found that the highest cytotoxic activity towards HepG2 cells was obtained after application of the hexane extract at IC_{50} of 93.17 µg ml^{-1} followed by acetone and diethyl ether extracts, while aqueous extract was effectiveness against HepG2 cell lines.

3.5. Cytotoxic activity of *S. obliquus* DEE fractions

Fractions F1 and F7 of *S. obliquus*, the fractions with the highest antibacterial and antifungal effect, were chosen for test its activities against cancer cell lines. The cytotoxicity of *S. obliquus* DEE fraction F1 against HepG2, HCT116 and MCF7 cancer cell lines is illustrated in Fig. 3. The highest anticancer activity was recorded against HepG2 cell lines with IC_{50} of 79.18 µg ml^{-1}, followed by breast cancer HCT116 and MCF7 cell lines with IC_{50} of 90.71 and 93.88 µg ml^{-1}, respectively. Fig. 4 shows the cytotoxicity of *S. obliquus* DEE fraction F7. The highest anticancer activity was shown against colon cancer cells CT116 at IC_{50} of 92.25 µg ml^{-1}, followed by breast cancer cells MCF7 with IC_{50} of 95.88 µg ml^{-1}. While, F7 had low activity against liver cancer cells HepG2 with IC_{50} of 114.53 µg ml^{-1}.

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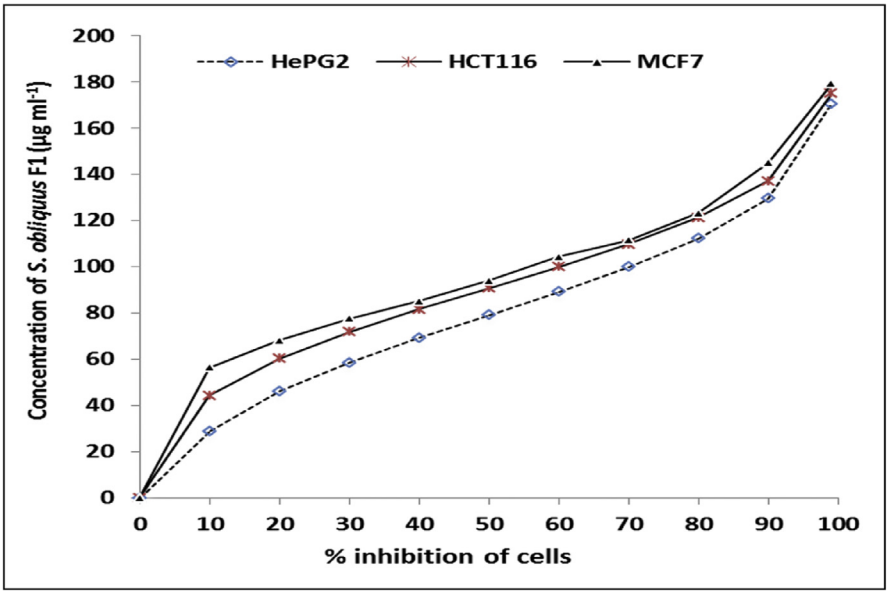

Fig. 3. Cytotoxic assay of *S. obliquus* DEE extract fraction F1 on HepG2, HCT116 and MCF7 cell lines.

Custódio *et al.* [31] reported that fatty acid fraction from hexane extract of *Scenedesmus* sp. had cytotoxicity against human hepatic carcinoma HepG2 cells with IC_{50} value of 0.11 mg ml^{-1}. Also, Abd El Baky *et al.* [10] found that fatty acids fractions of *S. Obliquus* had anticancer activity HepG2, HCT116 and MCF7 cancer cells. Dominguez-Bocanegra *et al.* [32] indicated that the lipophilic pigments astaxanthin, lutein and β-carotene from *Scenedesmus* sp. had effective anticancer activity.

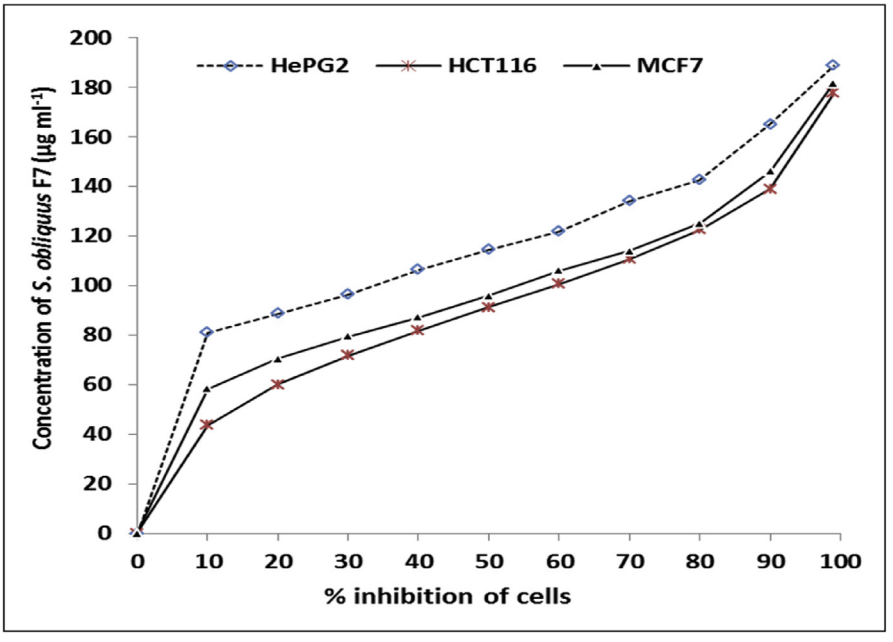

Fig. 4. Cytotoxic assay of *S. obliquus* DEE extract fraction F7 on HepG2, HCT116 and MCF7 cell lines.

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3.6. Identification of bioactive compounds using GC-MS

The results pertaining to GC-MS analysis of *S. obliquus* DEE fraction F1 and Mass Spectra of identified compounds in F1 and F7 are illustrated in Table 6 and Fig. 5. It revealed the presence of five compounds with retention time ranging from 10.45 to 39.26 min. The maximum peak was identified as nonadecane with peak area percent 76.10% followed by butylated hydroxytoluene 13.31% and 9-octadecenoic acid (Z) 6.97%. While, the minimum peak was shown as heptadecane 0.63% followed by hexadecane 1.28%. Seven compounds were identified by GC-MS from *S. obliquus* fraction F7. These compounds were 3-hexadecyloxycarbonyl-5-(2-hydroxyl)-4-methylimidazolium with peak area percent 3.57%, dodecanoic acid 3-hydroxy3.32%, 9-octadecenoic acid (Z)41.59%, 9,12,15-octadecadienoic acid 2.42%, 2-hexadecenal 5.11%, quercetin 7,3′,4′-trimethoxy 13.48%, octasiloxane 6.05% (Table 6).

Mubarak *et al.* [33] found that the fatty acid 9-octadecenoic acid from lipid extract of *Scenedesmus bijugatus* had antimicrobial activity against *S. aureus*, *E. coli* and *C. albicans*. Salem *et al.* [27] revealed that quercitin 7,3′,4′- trimethoxy from methanol fractions of *Scenedesmus* sp. showed antibacterial activity against *B. subtilis*, *K. pneumonia*e, *S. aureus* and antifungal activity against *F. solani, F. oxysporum, A. niger,* and *C. albicans*. Also, Marrez *et al.* [3] reported that quercetin 7,3′,4′-trimethoxy and octasiloxane from the cyanobacterium *O. brevis* diethyl ether fraction had broad spectrum antimicrobial activity against foodborne pathogenic bacteria and mycotoxigenic fungi. Shanab *et al.* [34]

Table 6. GC-MS analysis of components detected in fractions F1 and F7 of *S. obliquus* DEE extract.

No	RT	Compound	Area %		Molecular formula	MW
			F1	F7		
1	13.39	3-Hexadecyloxycarbonyl-5-(2-hydroxyl)-4-methylimidazolium	–	3.57	$C_{24}H_{45}N_2O_3$	409
2	17.25	Butylated hydroxytoluene	13.31	–	$C_{15}H_{24}O$	220
3	21.55	Dodecanoic acid 3-hydroxy	–	3.32	$C_{12}H_{24}O_3$	216
4	21.73	Nonadecane	76.10	–	$C_{19}H_{40}$	268
5	28.62	Hexadecane	1.28	–	$C_{16}H_{34}$	226
6	29.04	9-Octadecenoic acid (Z)-	6.97	41.59	$C_{18}H_{34}O_2$	282
7	34.64	9,12,15-Octadecadienoic acid	–	2.42	$C_{18}H_{30}O_2$	278
8	39.26	Heptadecane	0.63	–	$C_{17}H_{36}$	240
9	39.81	2-Hexadecenal	–	5.11	$C_{16}H_{34}O$	242
10	52.51	Quercetin 7,3′,4′-trimethoxy	–	13.48	$C_{18}H_{16}O_7$	344
11	56.68	Octasiloxane	–	6.05	$C_{16}H_{50}O_7Si_8$	578

https://doi.org/10.1016/j.heliyon.2019.e01404

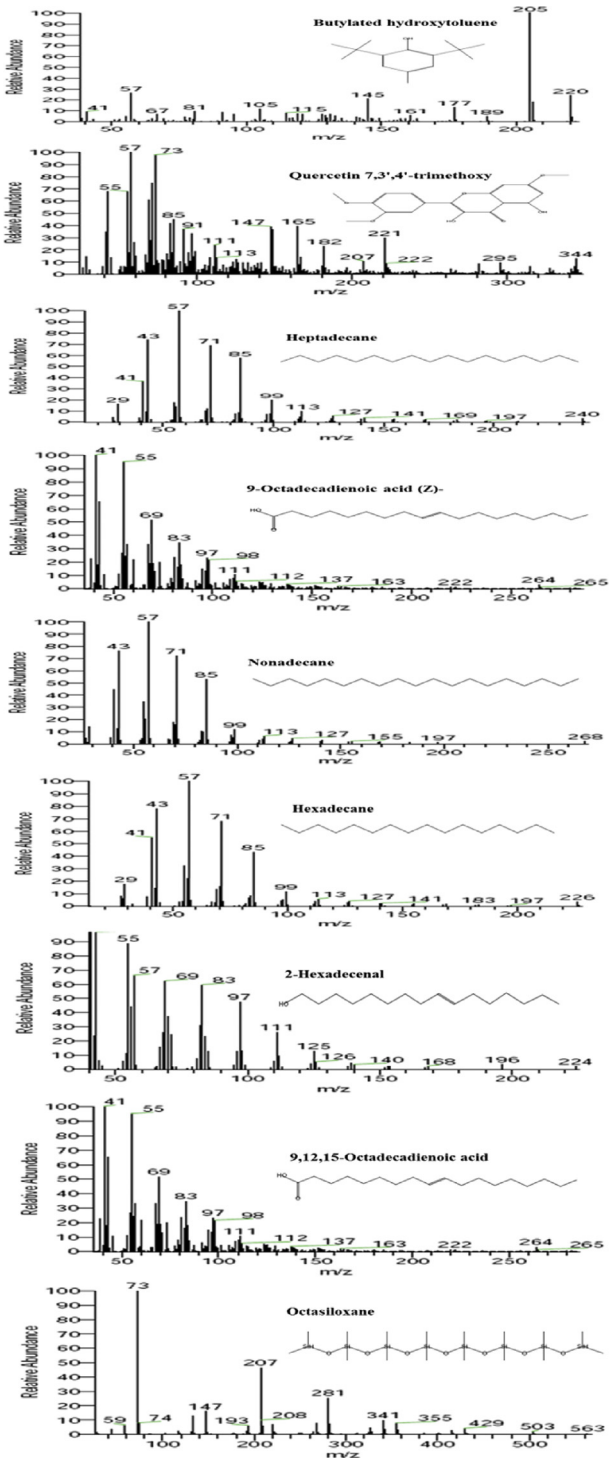

Fig. 5. Mass Spectra of identified compounds in F1 and F7.

https://doi.org/10.1016/j.heliyon.2019.e01404

reported that 9-octadecenoic acid (Z) and Dodecanoic acid 3-hydroxy which extracted from weed *Eichhornia crassipes* methanolic fractions showed antimicrobial activity against *B. subtilis*, *Streptococcus faecalis*, *E. coli*, *S. aureus*, *A. flavus* and *A. niger*. Marrez and Sultan [35] indicated that butylated hydroxytoluene isolated from *M. aeruginosa* had antifungal activity against several species of mycotoxigenic fungi.

Krishnaveni *et al.* [36] indicated that hexadecanoic acid, heptadecanoic acid and 9,12,15-octadecadienoic acid from oil of cotton seeds possessed antimicrobial activity against *E. coli*, *S. aureus*, *A. flavus* and *A. niger*. Mujeeb *et al.* [37] reported that 9-Octadecenoic acid, 2-hexadecenal and 9,12,15-octadecadienoic acid from leaves of *Aeglemarmelos* methanolic fractions had antibacterial activity against *K. pneumonia*e, *S. aureus* and *B. cereus*. Zoue *et al.* [38] indicated that octasiloxane from lipid fraction of *C. olitorius* and *H. sabdariffa* seeds had antimicrobial activity against *S. aureus*, *P. aeruginosa*, *E. coli* and *A. fumigatus*. Also, Ruba and Mohan [39] indicated that 3-hexadecyl oxycarbonyl-5-(2-hydroxyl)-4-methylimidazolium from ethanolic extract of *Andrographis echioides* had antimicrobial activity against several human pathogenic bacteria and fungi. Mavi *et al.* [40] reported that butylated hydroxytoluene from plant *Sonchus erzincanicus* methanolic extract had antimicrobial activity against *E.* coli, *P. aeruginosa*, *S. aureus* and *C. albicans*. Also, Demirel *et al.* [41] found that butylated hydroxytoluene from the essential oils of two coastal plants *L. obtusa* and *L. pyramidata* showed antimicrobial activities against *S. aureus*, *E. coli* and *C. albicans*.

Suriyavathana and Indupriya [42] reported that hexadecyloxycarbonyl-5-(2-hydroxyl)-4-methylimidazolium from ethanolic leave extract of *Blepharism aderaspatensis* showed anticancer activity against breast cancer cell lines MCF7 and colon cancer cell lines HCT116. Marrez *et al.* [3] found that *O. brevis* diethyl ether fraction contains quercetin 7,3′,4′-trimethoxy and octasiloxane exhibited anticancer activity against colon cancer HCT116 and breast cancer MCF7, while no activity was observed against hepatocellular carcinoma HepG2.

4. Conclusion

Scenedesmus obliquus diethyl ether extract was the extract that had the greatest biological activity against tested bacteria, fungi and cancer cell lines. Fractions F1 and F7 of diethyl ether extract effectively inhibited all tested microbes and cell lines. Eleven compounds were identified from F1 and F7, most of them have been reported to possess biological activities. The current study highlighted the potential application of *S. obliquus* as antimicrobial and anticancer agents.

https://doi.org/10.1016/j.heliyon.2019.e01404

Declarations

Author contribution statement

Diaa A. Marrez, Yousef Y. Sultan: Conceived and designed the experiments; Performed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Mohamed M. Naguib, Aziz M. Higazy: Conceived and designed the experiments; Analyzed and interpreted the data; Contributed reagents, materials, analysis tools or data; Wrote the paper.

Funding statement

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Competing interest statement

The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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