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Antioxidant And Anticancer Potential of Bioactive Compounds from Locally Isolated Microalgae

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ABSTRACT

The current cancer therapy research focuses on the curative strategies but also targeting to improve the development of the preventive strategies. Anticancer, antibacterial, antioxidant, and anti-inflammatory effects have been demonstrated by the bioactive compound extracted from microalgae. Marine organisms, particularly microalgae might be one the alternative that may contribute to the achievement of one of Sustainable Development Goals (SDG) which is SDG 3, Good Health and Wellbeing. It is crucial for long-term sustainable development to ensure healthy lives and promote well-being at all ages. Two microalgal samples from Malaysia *Acutodesmus obliquus* (CN01) and *Desmodesmus perforates* (SP04) were isolated from fresh waters and cultivated. A total of three different solvents, polar, intermediate and nonpolar solvent was used to extracts the microalgae sample which are methanol, acetone and hexane, respectively. *Acutodesmus obliquus* and *Desmodesmus perforates* extracts show low total phenolic content in range from 0.2447 ± 0.0009 to 0.2614 ± 0.0003. Secondly, the antioxidants activity of both microalgae shows significant radical scavenging activity especially in acetone extracts of both microalgae, with values of 11.5433 ± 0.0115 and 13.5847 ± 0.0618 for *Acutodesmus obliquus* (CN01) whereas *Desmodesmus* (SP04), 13.7898 ± 0.2601 and 22.3212 ± 0.2302 scavenging activity percentage. Then, the anticancer effect of the crude extracts was tested against breast cancer, MCF-7 breast cancer cell lines by using MTT assay and apoptosis assay. Methanolic extract has demonstrated high antiproliferative effect against MCF-7, with cell inhibition percentage within range of 87±1.1271 % to 73 ± 0.2744 % and 82 ± 0.0236 % to 73 ± 0.0423 % of *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04) respectively. Finally, both microalgae show similar compound group as HPLC is used to identified lipid, which is believed to play the big role in contributing to anticancer and antioxidant activity. This suggests that *Acutodesmus obliquus* sp and *Desmodesmus perforates* sp possess diverse biosynthetic capacities, potentially enabling the creation of new pharmaceuticals.

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1. Introduction

According to Globocan in 2020, cancer caused approximately 10 million deaths worldwide and 19,292,789 new cases were reported [1]. In Malaysia, cancer accounted for about 0.09% of the population's deaths, making it the leading cause of death in 2016, with 12.6% of deaths in government hospitals and 26.7% in private hospitals. The trend of cancer-related deaths in Malaysia increased from 11.3% in 2007 to 12.6% in 2016 [2].

Cancer occurs due to uncontrolled cellular proliferation [3], and current treatments using synthetic compounds are less efficient compared to natural compound-based drugs, which are easier to metabolize. Major treatments include alkylating agents and antimetabolites, which have significant side effects. Cancer can develop due to disruptions in cell death mechanisms, such as apoptosis, which is crucial for cancer therapy. The protein caspase plays a key role in apoptosis, and the tumor suppressor gene p53 helps prevent cancer. When p53 is mutated, its function is lost, leading to cancer formation [4].

Studies have shown that macroalgae and microalgae have potential anti-cancer properties, inducing apoptosis in cancer cells [5-7]. Microalgae, such as *Nannochloropsis*, contain various bioactive compounds like pigments, amino acids, and ω-3 fatty acids, which exhibit antitumor, antioxidant, and anti-inflammatory activities [8].

Up till today, cancer treatments conducted involve chemotherapeutic drugs, which cause severe side effects. Recent research focuses on natural ingredients from microorganisms, animals, and plants that target tumor cells without harming normal cells. Single-pathway treatments show limited success, necessitating combination therapies and advancements in precision medicine [9]. Nanomedicine can improve targeting but faces challenges like high interstitial fluid pressure and drug delivery issues in certain cancers [10]. Therefore, the extraction and analysis of bioactive compounds from natural sources should be considered as new alternative [11,12]. Plants and algae offer promising sources of anticancer agents with fewer side effects as compare to today treatment such as chemotherapy [13]–[15]. Algae, particularly microalgae, show potential in inducing cancer cell apoptosis without affecting normal cells, suggesting a need for more research into their use in cancer treatment [16]. For example, *Euglena tuba* extract has been effective against cancer cells while sparing normal cells [17].

There are over 200 types of cancer, which can spread to other tissues through metastasis, often resulting in death. Cancer develops due to damaged DNA, faulty DNA repair mechanisms, activation of tumors, and loss of tumor suppression functions [18]. Major causes of cancer include an unhealthy lifestyle, poor diet, aging, smoking, and environmental pollutants. High fatality rates often result from therapy failure and tumor relapse. During treatment, cancer cells may develop resistance due to improper pharmacological strategies or various drug resistance mechanisms such as drug efflux, detoxification, changes in drug targets, effective DNA repair, blocked apoptosis, and the development of resistant cancer stem cells [19].

Microalgae have significant potential for human health and medicine, with applications in cardiovascular health, cancer treatment, inflammation reduction, infection prevention, immune enhancement, and cholesterol reduction. They are also useful in treating tumors, stomach ulcers, and aiding wound healing, with components capable of detoxifying the body [20,21].

2. Methodology

2.1 Microalgae Cultivation

Microalgae culture for this study was obtained from an isolated strain sourced from the Algal Biomass Research Lab, *Acutodesmus obliquus* (CN01, MJIIT-UTM, Malaysia) and *Desmodesmus* (SP04, MJIIT-UTM). Since microalgae require specific nutrients for healthy growth, AF6 and BG-11 media were prepared for *Acutodesmus obliquus* sp and *Desmodesmus* sp respectively. Subsequently, the microalgae were allowed to grow under optimal conditions, exposed to white, fluorescent light at 80µmol photons/m2/s for a 12-hour photoperiod at 25°C, with continuous aeration. Regular monitoring of the microalgal culture for growth and changes in cell density was conducted using a UV-VIS spectrophotometer (Hach DR 6000, Hach, U.S.A.) at 750 nm optical density (OD). Harvesting of the microalgae occurred during the log phase, whereby harvested microalgae were stored at -80°C until further procedures were conducted. Subsequently, the frozen microalgae underwent freeze-drying using a freeze dryer (Scientz-10ND) to remove excess water. The resulting freeze-dried microalgae were stored at -80°C to preserve their viability until further use [23,24].

2.2 Solvent Extraction

5 grams of freeze-dried *A. obliqus* or *Desmodesmos* powder were agitated in 100 mL of hexane, acetone, or methanol for 24 hours, followed by filtration through cotton. The solvent was subsequently evaporated using a spin dryer at 35°C, and the resulting dried extract was stored at -20°C for future applications [25-27].

2.3 Measurement of Phenolic Content

The measurement of phenolic content of microalgae extract is done by using colorimetric assay. In this study, a suitable phenolic standard stock solution is prepared, with gallic acid serving as the chosen standard for analysis. Subsequently, a reaction mixture comprising crude extracts, Folin-Ciocalteu reagent, and sodium carbonate solution is meticulously prepared. Following preparation, the mixture is allowed to incubate for a duration of 30 minutes to facilitate the reaction process. After the incubation period, the absorbance of the resulting mixture is measured at 750nm using spectrometer UV-VIS spectrophotometer (Hach DR6000). Finally, the phenolic content of the microalgae extract is determined by calculating it based on the absorbance values obtained from the analysis, enabling a comprehensive assessment of the extract's phenolic composition [22].

2.4 Radical Scavenging Inhibition Activity

In this study, DPPH (2,2-diphenyl-1-picrylhydrazyl) assay method is used to evaluate the antioxidant content of microalgae. To initiate the antioxidant activity assessment, a 1mM DPPH solution is first prepared by dissolving 1.2mg of DPPH in 50ml of methanol within a volumetric flask. The absorbance of this solution is meticulously evaluated and adjusted to fall within the prescribed range of 0.65 ± 0.02 at 517nm. Following this, compounds extracted from the microalgae are diluted to the desired concentration range of 2mg/ml to 10mg/ml using methanol as the selected solvent. Subsequently, 0.8ml of the prepared DPPH solution is mixed with 0.2ml of the diluted microalgae extract in a 1ml centrifuge tube, and the resulting mixture is then incubated for a duration of 30 minutes at room temperature under dark conditions. After the incubation period, the absorbance of the reaction mixture is measured at a wavelength of 517nm by using UV-VIS spectrophotometer

(Hach DR6000). Same procedure is repeated from ascorbic acid standard. This method allows for the comprehensive assessment of the microalgae extract's radical scavenging potential, providing valuable insights into its antioxidant properties [28]. Utilizing the acquired absorbance values, the antioxidant activity of the microalgae extract is determined by applying the formula below Equation (1), which calculates the radical scavenging activity (RSA) as a percentage: where Abs_{DPPH} represents Absorbance value of standard DPPH solution, and Abs_{DPPH} represents Absorbance value of standard DPPH solution.

$$RSA\ \% = \left(\frac{Abs_{DPPH} - Abs_{sample}}{Abs_{DPPH}}\right) \times 100 \quad (1)$$

2.5 Cell Culture

MCF-7 is a breast cancer cell line that is used in vitro in this study. Sterile techniques and proper safety precautions were involved throughout the entire process to prevent any contamination as referred to [29]. The cell line MCF-7 is cultured using Minimum Essential Medium (MEM) supplemented with 10% fetal bovine serum (FBS), 1% L-glutamine, and 1% penicillin/streptomycin to support cell growth and proliferation. The cells are then incubated in a humidified incubator with 5% CO_2 at 37°C by using Astec, APC-50DR. Daily monitoring of cell growth is conducted using an inverted microscope (Olympus CKX41), and the medium is changed every 3-5 days or as needed to maintain optimal growth conditions.

2.6 MTT Assay

MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay is used in this study in which colorimetric assay is used to measure cell viability and proliferation in vitro. The experimental procedure begins by plating cells in a 96-well plate at a density of 1 x 10^5 cells per well. Following plating, the cells are incubated at 37°C in a humidified incubator with 5% CO_2 for approximately 48 to 72 hours until firm attachment to the plate is confirmed. Upon confirmation of cell attachment, the medium is aspirated from the wells and replaced with microalgae extract solutions of varying concentrations, while fresh medium serves as a negative control and DMSO acts as a positive control. Subsequently, the cells are further incubated for an additional 24 hours. Following this incubation period, the MTT solution is prepared by dissolving MTT in phosphate-buffered saline (PBS) at a concentration of 5 mg/ml. After the 24-hour incubation period, the extracts, medium, and DMSO are removed from the wells, and an equal volume of MTT solution is added. The cells are then incubated for 4 hours at 37°C in a humidified incubator with 5% CO_2. Following the incubation, the MTT solution is carefully aspirated, and DMSO is added to dissolve the formazan crystals formed by viable cells. Finally, the absorbance of the dissolved formazan is measured at 560 nm wavelengths using a microplate reader, GloMax® Discover (PromegaInstrument, 9700000313) allowing for the assessment of cell viability and potential effects of the microalgae extract [30]. The cell viability and proliferation of the cells is calculated using the absorbance values obtained and formula as shown below Equation (2). Where OD_{Sample} represents Optical density of sample, and $OD_{control}$ represents Optical density of control.

$$Cell\ viabilbity\ percentage\ (\%) = \frac{OD_{sample}}{OD_{control}} x100 \quad (2)$$

2.7 Phytochemical Identification using GCMS

The chemical components of the extracts from both microalgae were analyzed using GC-MS (The Agilent 7820A GC) featuring a fused silica capillary column (30 m × 0.25 mm × 0.25 µm, coated with DB-5) and an electron ionization (EI) operating at 70 eV. The injector temperature was maintained at 250°C. The oven temperature was programmed to start at 40°C for 1 minute and then increase at a rate of 3°C per minute until reaching 280°C. Helium served as the carrier gas at a flow rate of 1 mL/min. Identification of compounds was achieved by comparing their mass spectra with those in the National Institute of Standards and Technology (NIST) library [31].

2.8 Statistical Analysis

The experiments were conducted three or more times, and the results are presented as mean ± SD. Group differences were assessed using one-way ANOVA with GraphPad Prism 9.0 software, where significance levels were denoted as ****P <.0001.

3. Result and Discussion

3.1 Total Phenolic Content

Phenolic compounds are key antioxidants, stabilizing radicals by donating hydrogen atoms or electrons. The total phenolic content in microalgae was measured as gallic acid equivalent (GAE) to compare different strains. These compounds, significant for their scavenging potential due to hydroxyl groups, help microalgae mitigate oxidative stress [32-34]. Therefore, in this study two local microalgae are used to analyse the total phenolic content which is a crucial parameter for measuring the antioxidant potential of *Acutodesmus obliquus* CN01 and *Desmodesmus* SP04. Total phenolic compound is determined by using Folin-Ciocalteu method. Total phenolic compounds are measured using the Folin-Ciocalteu method, where microalgae extract react with Folin-Ciocalteu reagent to form a blue complex. The phenolic content is determined by measuring the colour intensity with a spectrophotometer, using a gallic acid standard curve.

Three different solvents with different polarity were used to evaluate the total phenolic content (TPC) which are methanol, acetone and hexane of two microalgae which are *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04). From Figure 1, *Acutodesmus obliquus* (CN01) has a total phenolic content of 0.2447 to 0.2522 mg/g GAE, while *Desmodesmus* (SP04) ranges from 0.2466 to 0.2614 mg/g GAE. The highest phenolic content is found in *Desmodesmus* methanolic extract (0.2614 ± 0.003 mg/g GAE), followed by *Acutodesmus obliquus* methanolic extract (0.2522 ± 0.0007 mg/g GAE). Hexane extracts show the lowest TPC values for both microalgae. Acetone extracts have higher TPC in *Desmodesmus*, 0.2487 ± 0.0001 mg/g GAE compared to *Acutodesmus obliquus*, 0.2472 ± 0.0003 mg/g GAE. Overall, TPC is highest in methanol extracts, followed by acetone, and lowest in hexane. This trend aligns with the polar nature of phenolic compounds, as seen in previous studies [32,35].

Comparing our analyzed strain of *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04), phenolic content is significantly lower compared to other previously published data. For example, green microalga *Scenedesmus* sp was observed to have the highest TPC in ethanol/water, ranging from 0.5 to 4.6 mg GAE g−1 compared to TPC to 0.2447 mg/g to 0.2522 mg GAE g−1 in *Scenedesmus* sp. It's crucial to acknowledge that various factors such as growth conditions, pH, temperature, light exposure, harvesting timing, alongside inherent variability and extraction techniques, influence the phenolic content and antioxidant capacities of microalgae [36]. A lot of factors play such a big role in extracting phenolics compound, as shown in Ramalingam Parthasarathy study, higher phenolic

compound of 3.4 mg/gae can be extracted when using hot water extraction method. Thus, explained that different methods and solvents do affect the phenolic content extracted.

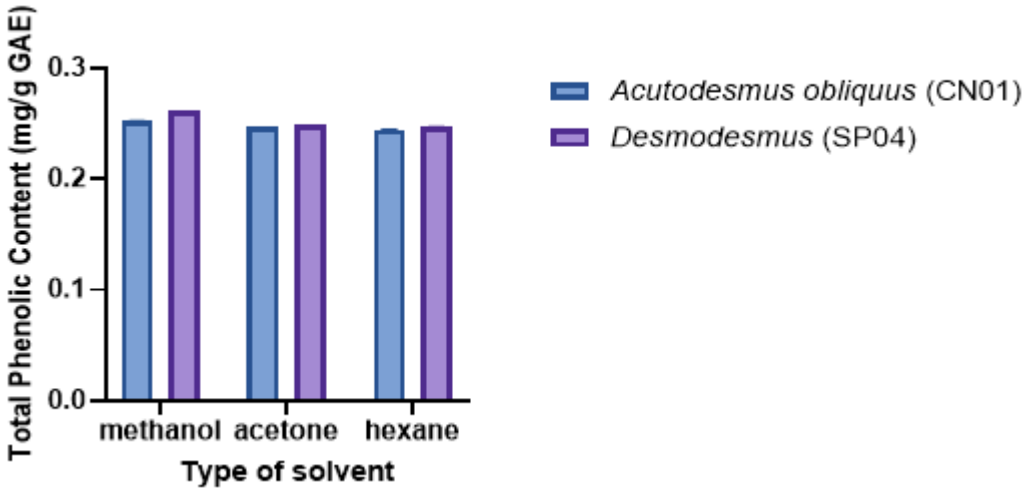

Fig. 1. Total Phenolic Content of *A. obliqus* and *Desmodesmus* in methanol, acetone and hexane. Expressed as mean ± standard deviation (SD) of triplicate sample extract

3.2 Radical Scavenging Activity

The DPPH assay evaluates the ability of tested antioxidants to reduce the DPPH· radical either through direct electron transfer or by quenching the radical via hydrogen atom transfer [37]. The solubility of antioxidants, primarily influenced by the polarity of the solvent employed, plays a crucial role in accurately evaluating antioxidant capacity. In this research, we utilized three solvents with differing polarities: methanol, acetone, and hexane for extraction.

The antioxidant potential of *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04), extracts was assessed through DPPH assays. Figure 2 and Figure 3 presents the findings, indicating the percentage of DPPH radical scavenging activity of microalgae. The results reveal that the highest antioxidant capacity, was observed in the acetone, with values of 11.5433 ± 0.0115 and 13.5847 ± 0.0618 for *Acutodesmus obliquus* (CN01) whereas *Desmodesmus* (SP04), 13.7898 ± 0.2601 and 22.3212 ± 0.2302 scavenging activity percentage. Extracts in other solvents demonstrated comparatively lower antioxidant capacities. Statistical analysis showed significant differences ($p < 0.0001$) between hexane, methanol, and acetone compared to ascorbic acid. Our findings align with prior research indicating that the selection of solvent significantly impacts the extraction yield of substances, as suggested by [35].

Between *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04), *Desmodesmus* (SP04), shown high significance scavenging activity compared to *Acutodesmus obliquus* (CN01) in overall as shown in Figure 2 and Figure 3. However, both microalgae do display the same pattern of which acetone exhibit higher scavenging activity than both methanol and hexane. conversely, the ethanolic extracts from *U. fasciata* and *G. salicornia* showed significant antioxidant activity. The antioxidant effects can be credited to phenolic compounds along with various other phytochemical compounds like carotenoids, polyunsaturated fatty acids (PUFA), and polysaccharides found within the algal extract, [22].

Martin Almendinger *et al.,* [38] discovered that the strains with the highest content of water-soluble compounds were measured between 12–14 µmol ascorbic acid equivalent per gram, while

N. oleobundans and *A. obliquus* were the strains exhibiting the highest concentrations of lipid-soluble compounds, ranging between 60–80 µmol trolox equivalent per gram. According to Amzah *et al.,* [39], this particular strain of *Acutodesmus obliquus*, also referred to as *Scenedesmus obliquus* or *Tetradesmus obliquus*, has been previously demonstrated to possess a significant number of fatty acids. Additional elements like the composition of fatty acids, could potentially enhance the antioxidant capacity of both *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04), as indicated [40,41].

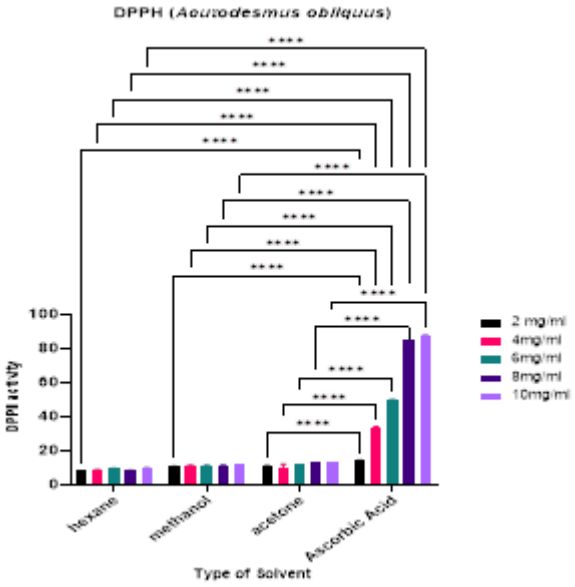

Fig. 2. DPPH radical scavenging activity of A*cutodesmus obliqus* of methanol, acetone, and hexane extracts. All result is expressed in mean ± SD. (p<0.0001) (n=3)

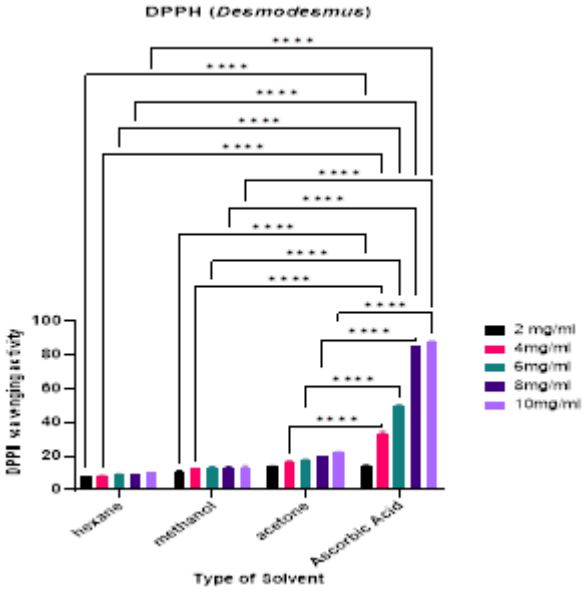

Fig. 3. DPPH radical scavenging activity of *Desmodesmus* of methanol, acetone, and hexane extracts. All result is expressed in mean ± SD. (p<0.0001) (n=3)

3.3 Anticancer Property

The MTT assay is used to evaluate cytotoxicity in MCF-7 cell cultures. It involves adding the MTT compound to cells, which is metabolized by living cells into insoluble formazan crystals. After a period of incubation, the cells are lysed to release the formazan, which is then solubilized and measured spectrophotometrically. The absorbance is directly proportional to the number of viable cells, allowing for the assessment of cytotoxic effects of substances or treatments on cell cultures [28,42].

The cytotoxicity impact of three solvent namely methanol, acetone and hexane extract of *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04) was examined on MCF-7 breast cancer cell line via the colorimetric MTT assay. Where DMSO is use a control standard to compare with the extracts. Both microalgae show same patter in which MCF-7 cell viability decreases as concentration of the extract increases, in other word, all extract shows the same pattern of dose dependent manner against MCF-7.

Based on Figure 4 and Figure 5, acetone extracts from both microalgae showed the lowest cytotoxicity on MCF-7 cells: 83 ± 1.0723% to 79 ± 0.6934% for *Acutodesmus obliquus* (CN01) and 81 ± 0.0473% to 76 ± 0.5271% for *Desmodesmus* (SP04). Methanol extracts recorded the highest cell inhibition: 87 ± 1.1271% to 73 ± 0.2744% for *Acutodesmus obliquus* (CN01) and 82 ± 0.0236% to 73 ± 0.0423% for *Desmodesmus* (SP04). *Desmodesmus* had a higher anti-proliferative effect on MCF-7 cells than *Acutodesmus obliquus* (CN01), especially in methanolic extracts. The anticancer effect of

the extracts follows the order: methanol > acetone > hexane. These findings align with studies by Elia et al. [43] showing acetone extracts of *Laurencia papillosa* having dose-dependent cytotoxic effects on MCF-7 cells, and by Goh *et al.,* [44] and Suh *et al.,* [45], demonstrating the anticancer properties of ethyl acetate extracts from various algae.

Whereas methanolic extracts of both microalgae shows significant anticancer effect on MCF-7 demonstrates the decrease in cell viability by 73 ± 0.2744 % and 73 ± 0.0423 % of 8 mg/ml *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04) respectively. Similar observations have been made in the methanol extracts obtained from *Dunaliella tertiolecta* were effective solely against the A2058 human melanoma cell line. Moreover, Nigjeh *et al.*, [46] shows that the impact of crude ethanol extracts derived from a native microalga, *Chaetoceros calcitrans*, on human breast cell lines (MCF-7) demonstrate significant cytotoxic properties and might play the role in inducing the mechanism of apoptosis. Differences in the cytotoxic impact of a particular algal species can be attributed to the selection of extraction solvent and its efficacy in extracting bioactive compounds, bioactive compound composition, alongside seasonal and geographical fluctuations influenced by environmental factors like temperature, light exposure, and salinity alterations [47,48].

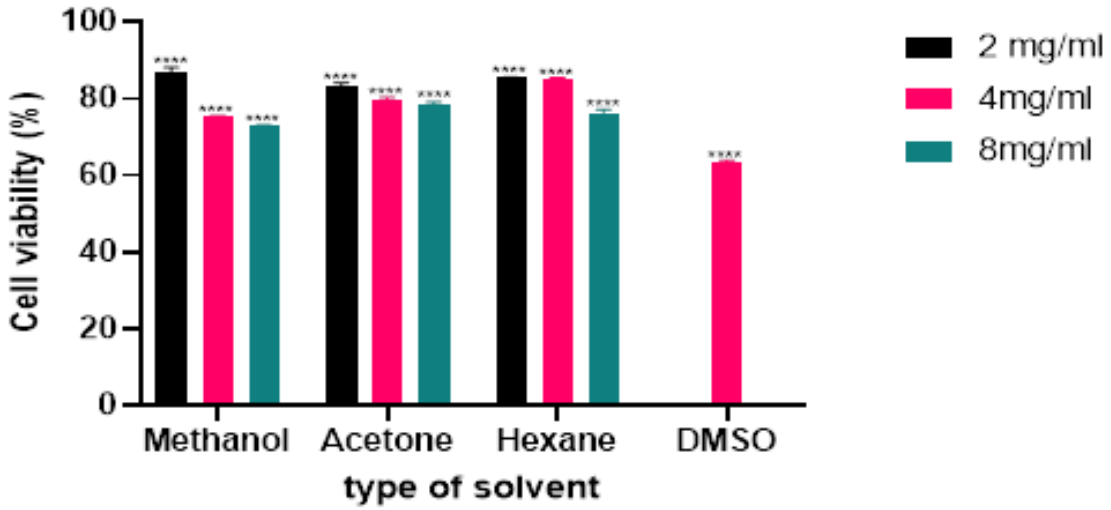

Fig. 4. Cytotoxicity of *Acutodesmus obliquus* (CN01) in human breast cell line MCF-7 cells, following incubation for 24 hours, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. All result is expressed in mean ± SD. ($p<0.0001$) (n=3)

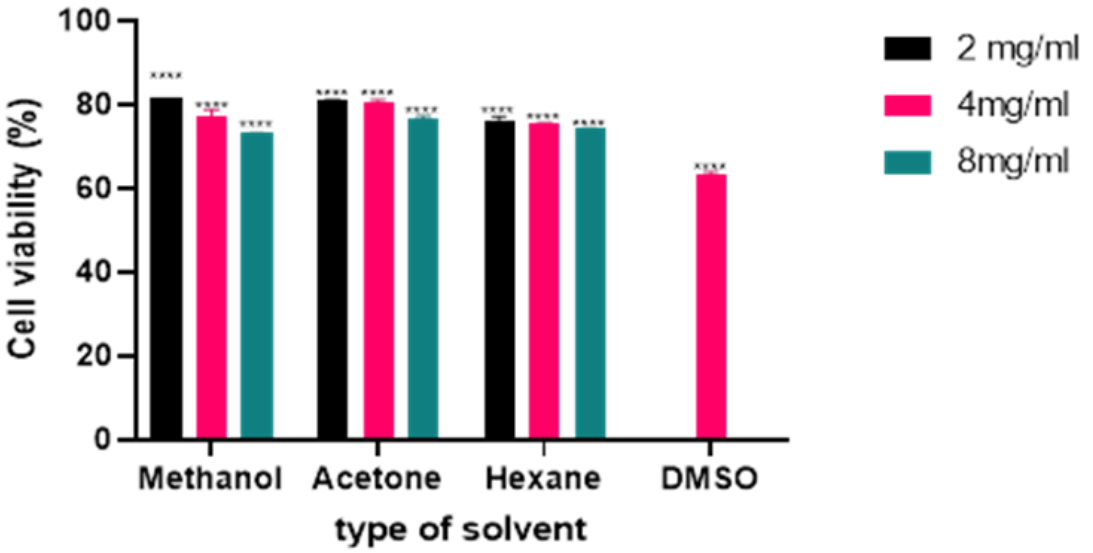

Fig. 5. Cytotoxicity of *Desmodesmus* (CN01) in human breast cell line MCF-7 cells, following incubation for 24 hours, using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. All result is expressed in mean ± SD. ($p<0.0001$) (n=3)

3.4 Phytochemical Analysis by GCMS

Two extracts out of six extracts have been chosen based on the previous antioxidant capacity and anticancer property, both methanolic extract of *Acutodesmus obliquus* (CN01) and *Desmodesmus* showing the most effective result in reducing the cell proliferation of MCF-7 breast cancer cell line. Therefore, further investigation of the phytochemical constituents is carried out using GCMS. As mentioned in the literature found, generally, green algae contain alkaloids, phenols, flavonoids, lipids, and polysaccharides which demonstrate anti-cancer properties and offer health benefits associated with antioxidants.

From GCMS result, we found that both freshwater green microalgae's major content is lipid. Fatty acids represent a significant lipid category present in macroalgae, serving as essential components of cell membranes such as phosphoglycerides or as reserves for energy for instance, triacylglycerol. Typically, they exhibit higher concentrations compared to other lipids such as sterols, hydrocarbons, and fatty alcohols. When comparing our findings with existing literature, it was noted that the predominant fatty acids detected in *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04) which are fatty acid, fatty acid methyl ester, fatty alcohol were consistent with those previously reported [49].

As referring to the finding tabulated in Table 1 and Table 2, the phytochemical found in the microalgae extracted, both *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04) was compared with previous studies. This shown that Dodecane and tetradecane from *Aspergillus niger* AK6 exhibit anticancer properties by inducing apoptosis and necrosis in MCF-7 cells, and also have antimicrobial and antifungal effects [50]. Dodecene from *X. testudinaria* shows anticarcinogenic effects on HeLa cells [51]. Metabolites like 1-Nonadecene, 1-Octadecene, and 1-Tetradecene in algae and plants combat cancer, act as antioxidants, and have antimicrobial properties [52]. Tetradecane in macroalgae contributes to antioxidant and antimicrobial effects [53]. *S. platensis* has tetradecane as a major component with antimicrobial activity [54]. Tetradecane from *Cladostephus spongiosus*

shows antioxidant properties [55]. Cyclotetradecane demonstrates antimicrobial [56] and antioxidant potential [57]. Tetradecane from *Ulothrix* sp shows antifungal activity.

Pacheco *et al.,* [58] reported that fatty acid extract of *A. utricularis* has antiproliferative activity on MCF-7 and MDA-MB-231 breast cancer cells. Hexadecanoic acid, or palmitic acid, enhances cytotoxic effects against cancer cells [59]. Octadecanol has antimicrobial and antioxidant properties [60].

GC-MS analysis of *Acutodesmus obliquus* (CN01) extract revealed bioactive compounds, including hexadecenoic acid, heptadecanoic acid, and octadecanoic acid. Lipid from *Chlorella sorokiniana* shows high cytotoxicity to A375, A549, and HeLa cells [61]. Hexadecanoic acid from *T. ornate* inhibits colon cancer cell proliferation [62]. Fatty acids from *C. silvatica* methanol extract reduce HeLa, PC3, and MCF7 cancer cell viability. Freshwater microalgae lipids have antimicrobial properties by damaging cell membranes [63].

9,12,15-Octadecatrienoic acid, methyl ester, from *Acutodesmus obliquus* (CN01), known as alpha-linolenic acid (ALA), is a precursor for n-3 polyunsaturated fatty acids (PUFAs) [64]. ALA exhibits potent antiproliferative effects on MCF-7 cells [65], reducing neoplastic transformation, inhibiting growth, and inducing apoptosis. ALA decreases cell viability and alters gene expression in various breast cancer cells [66]. ALA suppresses estrogen receptor-positive MCF-7 cell growth both in vitro and in vivo, likely by modulating ER-related signaling pathways [67]. These PUFAs may significantly contribute to the anticancer effect on MCF-7 cells in this research.

Table 1
Compounds found in methanolic extract of *Acutodesmus obliqus* (CN01) - Name, Retention Time (RT), and Peak area percentage of compounds present in the sample during GCMS analysis

Compound	Retention time (min)	Area %	Molecular weight (g/mol)	Molecular formula
7,10,13-Hexadecatrienoic acid, methyl ester	24.557	4.66	264.4	$C_{17}H_{28}O_2$
Hexadecanoic acid, methyl ester	25.046	11.28	270.5	$C_{17}H_{34}O_2$
Heptadecanoic acid, methyl ester	26.777	4.74	284.5	$C_{18}H_{36}O_2$
9,12-Octadecadienoic acid (Z,Z)-, methyl ester	28.020	4.46	294.5	$C_{19}H_{34}O_2$
9,12,15-Octadecatrienoic acid, methyl ester	28.223	15.19	292.5	$C_{19}H_{32}O_2$
Octadecanoic acid, methyl ester	28.556	4.01	298.5	$C_{19}H_{38}O_2$

Table 2
Compounds found in methanolic extract of *Desmodesmus (SP04)* - Name, Retention Time (RT), and Peak area percentage of compounds present in the sample during GCMS analysis

Compound	Retention time (min)	Area %	Molecular weight (g/mol)	Molecular formula
2-Dodecene	16.861	2.57	168.32	$C_{12}H_{24}$
Dodecane	21.944	5.03	170.33	$C_{12}H_{26}$
1-Tridecene	26.136	9.58	182.35	$C_{13}H_{26}$
Cyclododecane	30.496	8.48	168.32	$C_{12}H_{24}$
Tetradecane	30.825	4.16	198.39	$C_{14}H_{30}$
1-Pentadecene	34.639	6.77	210.40	$C_{15}H_{30}$
Pentadecane	34.947	3.15	212.41	$C_{15}H_{32}$
1-Octadecanol	42.310	1.96	270.5	$C_{18}H_{38}O$

4. Conclusions

The methanolic extracts of *Acutodesmus obliquus* (CN01) and *Desmodesmus* (SP04) demonstrated significant anticancer properties by effectively reducing the proliferation of MCF-7 breast cancer cells. GC-MS analysis revealed that these extracts are rich in bioactive lipids, including fatty acids, fatty acid methyl esters, and fatty alcohols, which are known for their anticancer, antioxidant, and antimicrobial properties. These findings align with existing literature, underscoring the therapeutic potential of green microalgae-derived compounds. This study indicated that these lipids play a crucial role in inducing apoptosis and inhibiting cancer cell proliferation through various cellular pathways. The identified compounds, such as dodecane, tetradecane, and alpha-linolenic acid (ALA), exhibited potent antiproliferative effects and contributed to the overall anticancer activity of the extracts. These bioactive lipids not only inhibit cancer growth but also modulate gene expression and cellular signalling pathways, enhancing their therapeutic efficacy. Future research should focus on comprehensive phytochemical profiling, in vivo validation, and comparative analysis with other anticancer agents. Additionally, exploring other microalgae species and developing advanced formulations can further enhance the stability, bioavailability, and targeted delivery of these bioactive compounds. By pursuing these avenues, we can unlock the full potential of green microalgae-derived compounds in cancer therapy and develop novel, effective anticancer agents.

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