Journal of Food, Nutrition and Diet Science
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Original Research

In vitro toxicity of microalgae species of the phyla chlorophyta and ochrophyta in CHO-k1 and HEP G2 cells for potential use in human nutrition

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Abstract: Microalgae are a promising component to enhance human nutrition, but to date only a few species have been authorized to be used in human nutrition. In this study, the in vitro toxicity of eight novel microalgae strains (Botryococcus braunii, Chlorococcum novae-angliae, Microchloropsis salina, Myrmecia bisecta, Stichococcus sp. Tetraselmis suecica, Tetradesmus obliquus and Spongiochloris minor) selected for their potential for human nutrition was investigated. N-hexane, acetone, ethanol and aqueous extracts of the lyophilized biomass were tested in the CHO-k1 and HEP G2 cell lines at concentrations of up to 1.81 mg ml^{-1} extracted biomass per well. None of the tested microalgae extracts reached values defined as a significant cytotoxic effect (IC_{50} < 0.02 mg ml^{-1}). The highest cytotoxic effects were measured for Stichococcus sp. in both cell lines with IC_{50} values of 0.17 mg ml^{-1} for the acetone extract in CHO-k1 cell culture and 0.18 mg ml^{-1} for the acetone extract in HEP G2 cell culture. Most cytotoxic effects occurred with the acetone and ethanol extracts, while the water and n-hexane extracts showed almost no measurable cytotoxic effects. Only Tetraselmis suecica showed no cytotoxic effect under the chosen conditions in both tested cell lines, marking this microalgae as particularly interesting for further investigations into its use in human nutrition.

Keywords: Microalgae, Cytotoxicity, in vitro toxicity, Human nutrition, Food safety

Received: Aug. 19, 2024; Revised: Oct. 12, 2024; Accepted: Oct. 21, 2024; Published: Oct. 25, 2024

DOI: https://doi.org/10.55976/fnds.220241297141-xx

Introduction

Microalgae are microscopically small algae [1]. Although they are all specified by their ability to perform oxygenic photosynthesis, they are a diverse, phylogenetically heterogenous group of microorganisms including approximately 72,500 species [1, 2]. Dietary supplements and functional foods containing microalgae have gained popularity in recent years. It is estimated that the global market for algae products will grow from USD 5.08 billion in 2023 to USD 7.09 billion in 2028 [3]. This indicates an increasing demand for microalgae supplements, which is justified by their wide range of nutritionally beneficial ingredients. They can synthesize high amounts of proteins, polyunsaturated fatty acids (PUFA), various vitamins and compounds with antioxidant capacities such as carotenoids and polyphenolic substances [4-6]. The most common species for human consumption, Chlorella sp. and Arthrospira sp. (known as "spirulina"), provide some of these benefits, but lesser known species such as the one discussed in this study covered may be even more suited. A study by Sangruber et al. (2023) demonstrated that smoothies containing Microchloropsis (M.) salina improved fatty acid distribution in plasma compared to smoothies containing Chlorella pyrenoidosa [7]. Although the range of microalgae products is steadily increasing, Chlorella sp. and Spirulina combined, account for more than 98 % of the microalgae produced worldwide [8]. Even though other microalgae species show high nutritional values as well [9]. Aphanizomenon flos-aquae, Haematococcus pluvialis, Ulkenia sp., Schizochytrium sp., Tetraselmis chuii and Odontella aurita are also approved for human nutrition in the EU [10]. The named species represent only a small fraction of the known microalgae [2]. It is quite conceivable that other microalgae that are not yet used for human consumption so far, may contain other or additional beneficial ingredients and therefore expand the spectrum for the use of microalgae [11, 12].

An important factor restricting the use of microalgae as food is their potential to synthesize toxins. Known microalgal toxins are microcystins, nodularin, anatoxin, saxitoxin and cylindrospermopsin. They are produced by cyanobacteria and dinoflagellates [13]. Additionally, some microalgae from the class Bacillariophyceae, known as diatoms, are known for being able to produce domoic acid, which can act as a neurotoxin [13-15]. The presence of known cyanotoxins in microalgae products already on the market has been investigated by different research groups. Most studies focus on dietary supplements containing cyanobacteria such as Spirulina or Aphanizomenon flos-aquae because cyanobacteria are potential toxin producers. Several researchers found microcystins and anatoxin-a in a variety of microalgae food supplements [16-20]. In addition, toxicity may also be caused by unknown components or metabolites of microalgae that do not belong to the groups mentioned [21]. To reduce the risks posed by toxins entering the food chain, the toxicity of novel food products is thoroughly tested before they are approved for human consumption [22]. This necessary but lengthy process is the main reason for the use of already established microalgae in food products. In this work, microalgae species that are currently not yet approved for use in human food were investigated for their cytotoxic potential to evaluate whether these microalgae could be promising candidates for future use in novel foods.

The eight selected species include Botryococcus (B.) braunii, Chlorococcum (C.) novae-angliae, M. salina, Myrmecia (M.) bisecta, Stichococcus sp. Tetraselmis (T.) suecica, Tetradesmus (T.) obliquus and Spongiochloris (S.) minor. They were selected for their potential to enrich human nutrition with PUFA, vitamins and polyphenolic compounds. B. braunii, C. novae-angliae, M. salina, M. bisecta and T. suecica can produce high amounts of eicosapentaenoic acid (EPA) and docosapentaenoic acid (DHA), which Chlorella sp. and Spirulina do not provide [23-29]. T. suecica and Microchloropsis sp. are also known to accumulate vitamin B12 in addition to their high PUFA content [30]. Stichococcus sp. and T. obliquus can accumulate vitamin B12 as well [30]. Commercially available microalgae such as Chlorella sp. can theoretically also accumulate vitamin B12, but studies with Chlorella sp. supplements show that the declared vitamin B12 concentrations are seldomly correct [29, 31]. Spirulina mainly produces pseudo cobalamin and is therefore not suited to supplement vitamin B12 in human nutrition [31]. T. obliquus can also produce high quantities of proteins and PUFAs, especially linoleic and linolenic acids [32, 33]. While proteins and PUFAs can also be supplemented by spirulina, the amount of n3-PUFAs is significantly higher in T. obliquus [29, 34]. The microalga S. minor was selected due to the amounts of polyphenolic compounds found in this species [35]. Polyphenolic compounds are known for their antioxidant potential, which makes them interesting additions to human nutrition [6, 36, 37]. Furthermore, all eight chosen species were evaluated for nutrients beneficial for human nutrition by Sandgruber et al. (2023) [34]. In this study, all eight species showed beneficial properties, with M. salina having the most benefits of the chosen species [34]. All eight species belong to the phyla Chlorophyta and Ochrophyta. So far, these phyla are not known to produce typical microalgal toxins.

Cytotoxicity was tested against two different cell lines. The CHO-k1 cell line is a rodent cell line known for its use in the evaluation of cytotoxicity and genotoxicity of novel foods and pharmaceuticals [38-40]. CHO cells are recommended in the OECD guideline 487 [41]. This OECD guideline is also referenced to by the European Food Safety Authority in its paper on genotoxicity testing for food and feed safety assessment [42]. HEP G2 cells are a human hepatocellular carcinoma cell line that was chosen as they are often used for screening cytotoxic or hepatoprotective substances [43-45].

Our study aimed to find out which of these microalgae do not exert significant cytotoxic effects on the cell lines we

selected in order to gain an initial understanding of their suitability for human consumption. This is particularly important for those microalgaes that produce EPA and DHA, as these nutrients are not readily available except from fish [46, 47]. For this reason, it is important to find new sustainable sources of EPA and DHA, which the tested microalgae could provide [48].

Material and Methods

Chemicals

Iscove's modified Dulbecco's medium (IMDM) with HEPES and L-glutamine, Ham's F12 medium with L-glutamine, RPMI 1640 with L-glutamin, penicillin-streptomycin mixture (5000 units ml^{-1} penicillin, 5000 µg ml^{-1} streptomycin) and trypsin/EDTA (10X) (contains 5 g L^{-1} trypsin 1:250 and 2 g L^{-1} Versene® (EDTA)) were obtained from Lonza (Verviers, Belgium). Heat-inactivated fetal bovine serum (FBS) was obtained from Life Technologies GmbH (Darmstadt, Germany).

For cytotoxicity measurements, Rotitest®Vital (2-(2-methoxy-4-nitrophenyl)-3-(4-nitrophenyl)-5-(2,4-disulfophenyl)-2H-tetrazolium) (WST 8)) was supplied by Carl Roth (Karlsruhe, Germany). Solvents for the extraction of algal biomass such as acetone (ROTISOLV® HPLC), ethanol (ROTISOLV® HPLC Gradient Grade), n-hexane (ROTISOLV® HPLC) and dimethyl sulfoxide (DMSO) (ROTIPURAN® ≥ 99.8 %) were obtained from Carl Roth (Karlsruhe, Germany) as well.

Microalgae strains and biomass production

The eight microalgae strains tested are listed in Table 1. They were cultivated at the Competence Center Algal Biotechnology of the Anhalt University of Applied Sciences in Köthen, Germany. The microalgae biomass was produced in 1.5 L bubble column photobioreactors or 10 L flasks. The culture media are also specified in Table 1. Cultivation was performed in duplicates (2-12 replicates) and cultures were illuminated with 100 or 300 µmol photons m^{-2} s^{-1} by LED panels or neon tubes, respectively, with a 14 h/10 h light/dark cycle, at room temperature (21-26°C) and aerated with CO2-enriched air (1.67 × 10^{-5} m^3 s^{-1}, 1% CO2). The initial concentration of biomass was adjusted between 0.1 to 0.5 g L^{-1}.

The cultivation was maintained until the stationary phase was reached (5-33 days). The biomass was harvested by centrifugation at 16,000 × g using a Thermo Multifuge X3R (Thermo Scientific, Germany) equipped with an F146 × 250 rotor with 6 × 250 mL centrifuge cups. The harvested biomass was washed twice. For mechanical disruption of the algae cell walls, a biomass slurry with 10 % dry matter was prepared in water, cooled on ice and continuously fed into a MiniSeries laboratory agitator bead mill (Netzsch, Germany) until the complete disintegration of the algae cells could be observed under the microscope. The agitator bead mill was operated at a milling speed of 3000 min^{-1} using 0.5 mm ZetaBeads (Netzsch, Germany) as the grinding medium, with which the grinding chamber was 80 % filled. The disintegrated biomass was lyophilized using a laboratory freeze dryer Beta 2-8 LD Plus (Martin Christ, Germany). The lyophilized biomass was stored at -20°C.Preparation of extracts

Table 1. List of investigated microalgae species including specification of culture medium, cultivation vessel and illumination [14]

Species name	Strain	Phylum	Class	Order	Family	Source	Medium	Cultivation vessel	Illumination (µmol photons m^{-2} s^{-1})
S. minor	KASC 29-01	Chloro phyta	Chloro phyceae	Chlamy domonadales	Chloroco ccaceae	Terrestrial	BBM	10 L flask	300 (Neon tube)
C. novae-angliae	SAG 5.85	Chloro phyta	Chloro phyceae	Chlamy domonadales	Chloroco ccaceae	Freshwater	ES	10 L flask	300 (Neon tube)
B. braunii var. Showa	University of Tokyo, Prof. Dr. Shigeru Okada	Chloro phyta	Trebouxio phyceae	Trebouxiales	Botryoco ccaceae	Freshwater	BG11	1.5 L bubble column	100 (LED Panel)
M. bisecta	SAG 2043	Chloro phyta	Trebouxio phyceae	Trebouxiales	Trebouxiaceae	Terrestrial	BBM	1.5 L bubble column	100 (LED Panel)
T. obliquus	SAG 276-1	Chloro phyta	Chloro phyceae	Sphaero pleales	Scenedes maceae	Freshwater	BBM	10 L flask	300 (Neon tube)
Stichoco ccus sp.	KASC 30-01	Chloro phyta	Trebouxio phyceae	Prasiolales	Stichoco ccaceae	Freshwater	BBM/ Setlik (1:1)	1.5 L bubble column	100 (LED Panel)
T. suecica	CCAP 66/38	Chloro phyta	Chlorodendro phyceae	Chloroden drales	Chloroden draceae	Marine	SWES	1.5 L bubble column	100 (LED Panel)
M. salina	SAG 40.85	Ochro phyta	Eustigmato phyceae	Eustig matales	Monodop sidaceae	Marine	F/2	10 L flask	300 (Neon tube)

Preparation of extracts

Biomass extraction was carried out as described by Himuro et al. (2017) with the following modification: in addition to ethanol and water, hexane and acetone were used as solvents to extract components of different polarity [49]. An ultrasound processor (200 W, 24 kHz) (Dr. Hielscher GmbH, mod. UP200S, Teltow, Germany) with a titanium horn tip of 2mm diameter (Dr. Hielscher GmbH, mod. S26d2, Teltow, Germany), equipped with an amplitude and pulse control system, was used for sonification at an amplitude of 190 µm (100 % amplitude, 600 W/cm^2) and a pulse cycle of 0.6. The ultrasonic treatment was carried out for 1 minute on ice. The extraction was scaled down to the use of 10 mg of microalgae dry matter for extraction with hexane, acetone and ethanol. The extracts were concentrated and redissolved in 0.5 ml of solvent or 0.5 ml of a mixture of the solvent and 10 % (v/v) DMSO, resulting in a final concentration of 20 mg extracted biomass ml^{-1}. Thereby the solubility of the extracted components was ensured under the test conditions.

For ethanolic extraction, aliquots containing 10 mg of microalgal dry matter per 1.5 ml ethanol (100 %) were prepared and sonicated on ice for one minute. The sonicated samples were stored in the dark at 25 °C for 24 hours and then centrifuged at 15,285 × g for 20 minutes. The supernatant was transferred into a new tube and the ethanol was evaporated to dryness in a vacuum concentrator (Eppendorf, mod. Concentrator 5301, Hamburg, Germany) for 60-90 minutes. The dry residue was redissolved in 0.4 ml of 10% (v/v) DMSO and 0.1 ml of 80% (v/v) ethanol. Extraction with acetone and n-hexane followed a similar protocol, except that the aliquots contained 10 mg dry matter per 1.5 ml acetone (100 %) and n-hexane (100 %), respectively, and the final solvent consisted of 0.4 ml of 10 % (v/v) DMSO and 0.1 ml of 100 % acetone or 0.5 ml of 100% n-hexane without DMSO, respectively. The aqueous extraction was conducted according to Himuro et al. (2017), but scaled down to 50 mg of microalgae dry matter [49].

All extracts had a final concentration of 20 mg extracted biomass ml^{-1} extracts and were serially diluted four times with 10 % (v/v) DMSO for ethanol and acetone extracts, with 100 % n-hexane for hexane extracts and with distilled, sterilized water for aqueous extracts (one part extract: 2.5 parts solvent). The dilution resulted in concentrations of 5.71, 1.63, 0.47, and 0.13 mg extracted biomass ml^{-1} in the cell culture assay wells. These concentrations were chosen based on preliminary experiments (not published) to include at least one non-cytotoxic concentration for all chosen algae to ensure the plateau could be reached.

Cell culture and exposure to extracts

The four extracts of eight microalgae species were tested against two cell lines. CHO-k1 cells (Chinese hamster ovary cells) were kindly provided by the collection of cell lines in veterinary medicine, Greifswald, Germany (CCLV-RIE 315). They were cultured in IMDM/F12 supplemented with 10 % FBS and antibiotics (100 units ml^{-1} penicillin. 100 µg ml^{-1} streptomycin). HEP G2 cells (hepatocellular carcinoma cells) (DSMZ number: ACC 180) were cultured in RPMI 1640 supplemented with 10 % FBS and antibiotics (100 units ml^{-1} penicillin, 100 µg ml^{-1} streptomycin). Both cell lines were cultivated in a humidified incubator at 37 °C and a 5 % CO2 atmosphere.

Cytotoxicity assay (WST-8-Assay)

Cytotoxicity was assessed for each algae extract and cell line using the WST-8 cell viability assay (Rotitest®Vital, Carl Roth GmbH + Co. KG, Karlsruhe, Germany) according to the instructions for use. This colorimetric assay is based on the reduction of the water-soluble tetrazolium salt to orange formazan dye, which absorbs light at 460 nm by NADH/NADPH-dehydrogenase.

For cytotoxicity assessment, 100 µl of CHO-k1 and HEP G2 cells were seeded into 96 well plates at a density of 5*103 cells per well, and cultivation was continued for 24 hours (CHO-k1 cells) or 72 hours (HEP G2 cells) in a humidified incubator at 37 °C in a 5 % CO2 atmosphere until subconfluency was reached. The cells were incubated for another 24 hours with 10 µl of either the prepared extracts, sterilized and distilled water (negative control for aqueous extracts), n-hexane (negative control for hexane extracts), ethanol or acetone diluted with 10 % (v/v) DMSO in water (negative controls for ethanol and acetone extracts) or 1 % (v/v) Triton-X-100 (positive control for all extracts) (Table 2). Then 10 µl of the WST-8 test solution was added to each well and the absorbance was measured with a microplate reader (Tecan Group Ltd., mod. Sunrise-basic, Männedorf, Switzerland) directly after adding the WST-8 test solution (t = 0) and a second time after 3 hours (t = 180) according to the manual.

The wavelength used for measuring absorbance was 450 nm with a reference wavelength of 620 nm. Three wells were used for each concentration and each control. All experiments were performed in four independent determinations on different days and in different passages (n = 4). To verify that the effects shown in the assay were cytotoxic rather than antiproliferative, the wells were also observed microscopically using an inverse microscope (Carl Zeiss AG, mod. Axiovert 40C, Jena, Germany).

Statistical analysis

The final absorption of each well was defined as Abs = $Abs_{t = 180}$ - $Abs_{t = 0}$ and expressed as a percentage of the respective negative control for each extract. By subtracting Abst=0, the different absorbance of the microalgae samples was negated. A dose-response curve was obtained for each algae extract tested. The concentrations of algae extract that inhibited 25 % (IC_{25}), 50 % (IC_{50}), and 75 % (IC_{75}) of the cell population were calculated with a nonlinear

Table 2. Concentration of used solvents and controls for each extract

Algal Extract		Negative Controls for each extract		Positive Control	
Solvent	Concentrations (% (v/v) in cell culture)	Solvent	Concentrations (% (v/v) in cell culture)	Chemical	Concentration (% (v/v) in cell culture)
Hexane-n	1.81; 0.51; 0.15; 0.04; 0.012	Hexane-n	9.09	Triton-X-100	0.91
Acetone	1.81; 0.51; 0.15; 0.04; 0,012	Acetone	1.81; 0.52; 0.15; 0.04; 0.01	Triton-X-100	0.91
Ethanol	1.81; 0.51; 0.15; 0.04; 0.012	Ethanol	1.45; 0.42; 0.12; 0.03; 0.01	Triton-X-100	0.91
Water	1.81; 0.51; 0.15; 0.04; 0.012	Water	9.09	Triton-X-100	0.91

regression model (using log transferred data). The IC_{25}, IC_{50} and IC_{75} were expressed in mg extracted biomass ml^{-1}. If one or more ICs were not included in the experimental data, the IC value was estimated using the nonlinear regression model (indicated as *estimated value). For this, the nonlinear regression curve fitted to the algae was expressed as a function and solved for Y = 25, Y = 50 and Y = 75.

Some extracts showed no cytotoxicity in the tested concentration range, so it was not possible to calculate IC_{25}, IC_{50} or IC_{75} values. If all four determinations showed no cytotoxicity in the tested range, the data was indicated as not calculable (nc). If one or more of the replicates showed cytotoxicity, the dose-response curves were established and analyzed by nonlinear regression for each independent determination and the mean and standard error were calculated. All calculations were done with graph pad prism software (GraphPad Prism Version 4.00).

According to Niccolai et al. (2017), the threshold for cytotoxicity was defined as an IC_{50} of more than 15 mg extracted biomass ml^{-1} and therefore any IC_{50} above 15 mg extracted biomass ml^{-1} was expressed as > 15 mg extracted biomass ml^{-1} [50]. This value represented the highest threshold for cytotoxicity found in all studies reviewed and was chosen to screen the microalgae for their safety for human consumption.

Results

The IC_{50} of all eight microalgae species extracted with four different solvents are shown in Figure 1 for the CHO-k1 cell line and in Figure 2 for the HEP G2 cell line. Only the biomass of T. suecica displayed an IC_{50} > 15 mg ml^{-1} for all tested extracts in both cell lines and was classified as non-cytotoxic according to Niccolai et al. (2017) [50]. All other microalgae species showed an IC_{50} < 15 mg ml^{-1} for at least one tested extract in one cell line (Table S1) (Figure 1 - 2).

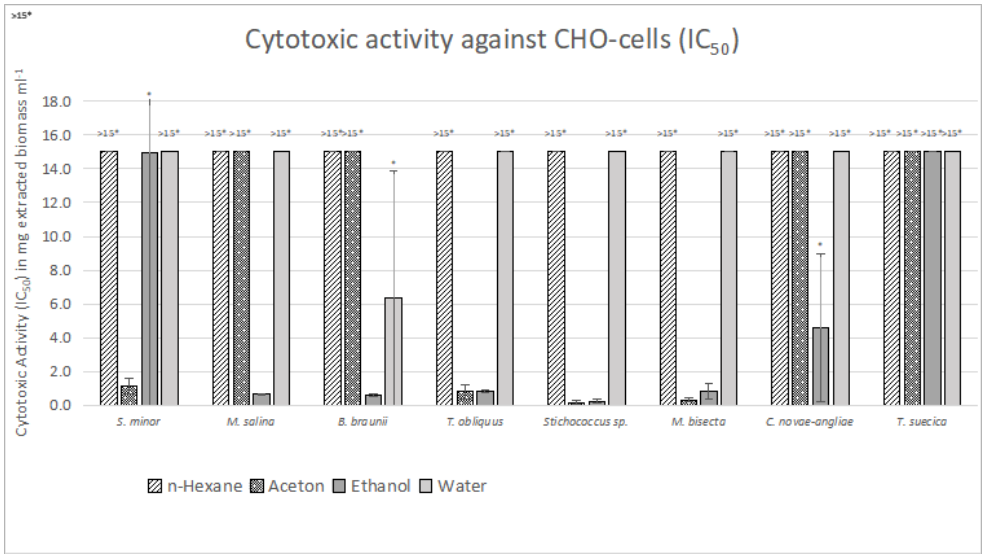

Figure 1. Cytotoxic activity (IC_{50}) expressed as mg extracted biomass ml^{-1} of different microalgae species against CHO-cells; * = estimated value; > 15 = values above 15 mg extracted biomass ml^{-1}

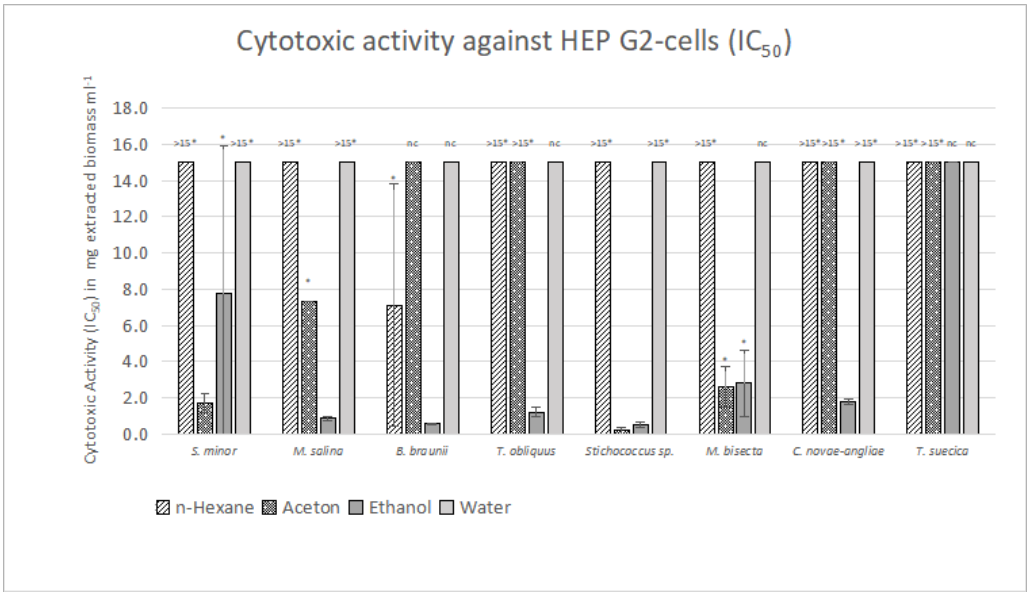

Figure 2. Cytotoxic activity (IC_{50}) expressed as mg extracted biomass ml^{-1} of different microalgae species against HEP G2 cells; nc = not calculable; * = estimated value; > 15 = values above 15 mg extracted biomass ml^{-1}

For B. braunii, the cytotoxicity of the aqueous extracts was demonstrated in CHO-k1 cells and the of hexane extracts in HEP G2 cells. All ethanolic extracts tested, except T. suecica, showed cytotoxic effects in both cell lines, ranging from IC_{50} values of 14.92 mg ml^{-1} (CHO-k1) and 7.73 mg ml^{-1} (HEP G2) in S. minor to 0.27 mg ml^{-1} (CHO-k1) and 0.52 mg ml^{-1} (HEP G2) in Stichococcus sp. extracts (Table S1).

For most extracts, the cytotoxicity was similar in CHO-k1 and HEP G2 cells. Only in B. braunii, M. salina and T. obliquus did the cytotoxicity differ noticeably between these two cell lines (Figure 1 - 2). For M. salina, the acetone extract displayed cytotoxicity with an estimated IC_{50} of 7.31 mg ml^{-1} in one of four independent determinations in HEP G2, but not in CHO-k1 cells. For T. obliquus, an IC_{50} of 0.81 mg ml^{-1} for the acetone extract in CHO-k1 cells and an IC_{50} >15 mg ml^{-1} in HEP G2 cells was calculated (Table S1). In B. braunii, cytotoxic effects on HEP G2 cells could be detected in the n-Hexane extract but not in the aqueous extract, while it was the other way around in CHO-k1 cells.

The differences in toxicity could be demonstrated more clearly when the IC_{25} and IC_{75} values are included in the evaluation (Table 3 - 4). The strongest cytotoxic effects for ethanolic and acetone extracts in CHO-k1 and HEP G2 cells were reached by Stichococcus sp. (Figure 1 - 2). M. salina and B. braunii also showed high cytotoxicity in ethanolic extracts, but no or weak cytotoxicity in acetone extracts to both cell lines (Figure 1 -2). In M. bisecta, the toxicity of acetone and ethanolic extracts to both cell lines seemed similar when only considering the IC_{50}. When looking at the IC_{75}, the divergence of the cytotoxicity was more pronounced for the different cell lines and the cytotoxicity in HEP G2 cells was weaker for both extracts (IC75 >15 mg ml^{-1}) than cytotoxicity for CHO-k1 cells (Table 3-4).

In general, it could be demonstrated that the tested aqueous and hexane extracts had a calculated IC_{50} > 15 mg ml^{-1} or no toxicity in the tested range in all but one case (Table S1), while most cytotoxic effects were observed in ethanolic extracts. The effects on CHO-k1 cells were slightly more pronounced than the effects on HEP G2 cells (Table S1; Table 3-4).

Discussion

Screenings for cytotoxic algae components were performed to test cytotoxicity against either cancer or non-cancerous cell lines [51, 52]. Antimicrobial, antifungal, antiviral or anticancer activities are of interest for the pharmaceutical use of microalgae extracts, while cytotoxicity against non-cancerous cell lines can be a first step to assess the safety of microalgae species [52-54, 50]. This paper aims to present the first toxicity screening for eight microalgae of interest as a food source. The final assessment of safety for human consumption still rely on in vivo methods, as substances that are cytotoxic in vitro may be non-toxic in vivo and vice versa. When assessing cytotoxicity, the IC_{50} values defined as significant cytotoxic effects vary, because any substance can be administered at a high enough dose to produce cytotoxic effects. Niccolai et al. (2017) defined a non-cytotoxic extract as an IC_{50} value above 15 mg extracted biomass ml^{-1} [50]. In contrast, a significant cytotoxic effect was defined as less than 0.02 mg extract ml^{-1} or less than 0.1 mg extract ml^{-1} when searching for anticancer drugs [55, 56]. It should be noted that the approaches in the studies differ in whether they calculate the IC_{50} of the amount of extract or the IC_{50} of the amount

Table 3. IC_{25} Values (expressed as mg extracted biomass ml^{-1}) calculated or estimated for microalgae extracts; data are reported as mean ± standard error; nc = not calculable; * = estimated value

Strain	IC_{25} Value (mg extracted biomass ml^{-1})							
	CHO-k1				HEP G2			
	n-Hexane	Aceton	Ethanol	Water	n-Hexane	Aceton	Ethanol	Water
S. minor	> 15*	0.71 ± 0.17	1.00 ± 0.20	1.54 ± 0.76	7.95 ± 4.90 *	0.74 ± 0.40	1.07 ± 0.73	11.36 ± 11.18 *
M. salina	> 15*	3.10 ± 1.76 *	0.52 ± 0.02	0.16 ± 0.11	>15*	3.51 *	0.50 ± 0.04	>15*
B. braunii	> 15*	0.22 ± 0.07	0.52 ± 0.04	0.91 ± 0.82	1.59 ± 0.58	nc	0.43 ± 0.01	nc
T. obliquus	> 15*	0.55 ± 0.26	0.68 ± 0.04	0.22	>15*	1.09 ± 0.34	0.73 ± 0.10	nc
Stichococcus sp.	2.75 ± 2.36*	0.13 ± 0.05	0.19 ± 0.05	0.66 ± 0.16	>15*	0.13 ± 0.06	0.38 ± 0.07	0.90
M. bisecta	> 15*	0.07 ± 0.02	0.58 ± 0.23	5.45	4.51 *	0.85 ± 0.32	0.76 ± 0.27	nc
C. novae-angliae	> 15*	8.76 ± 7.78 *	1.20 ± 1.00	13.62 ± 13.09 *	>15*	>15*	1.57 ± 0.07	0.13
T. suecica	8.54 ± 5.32 *	3.05	> 15*	> 15*	8.97 ± 5.32 *	>15*	nc	nc

Table 4. IC_{75} Values (expressed as mg extracted biomass ml^{-1}) calculated or estimated for microalgae extracts; data are reported as mean ± standard error; nc = not calculable; * = estimated value

Strain	IC_{75} Value (mg extracted biomass ml^{-1})							
	CHO-k1				HEP G2			
	n-Hexane	Aceton	Ethanol	Water	n-Hexane	Aceton	Ethanol	Water
S. minor	> 15*	2.24 ± 1.12 *	> 15*	> 15*	>15*	9.67 ± 7.35 *	>15*	>15*
M. salina	> 15*	> 15*	0.86 ± 0.04	> 15*	>15*	>15*	1.51 ± 0.06	>15*
B. braunii	> 15*	> 15*	0.79 ± 0.03	> 15*	>15*	nc	0.75 ± 0.06	nc
T. obliquus	> 15*	1.55 ± 0.40	1.10 ± 0.04	> 15*	>15*	>15*	1.95 ± 0.19 *	nc
Stichococcus sp.	> 15*	0.22 ± 0.05	0.38 ± 0.05	> 15*	>15*	0.26 ± 0.11	0.73 ± 0.08	>15*
M. bisecta	> 15*	2.29 ± 1.51 *	1.36 ± 0.28	> 15*	>15*	>15*	>15*	nc
C. novae-angliae	> 15*	> 15*	> 15*	> 15*	>15*	>15*	1.98 ± 0.08 *	>15*
T. suecica	> 15*	> 15*	> 15*	> 15*	>15*	>15*	nc	nc

of extracted algae biomass. With the latter approach, it is possible to compare two studies with different algae inputs, whereas this is not possible when cytotoxicity is calculated based on the amount of extract used. We used the approach adopted by Niccolai et al. (2017) and Himuro et al. (2017) [49, 50]. A comparison to the IC_{50} of specific extracts is therefore inconclusive. IC_{50} values are calculated values, which are estimated from the appropriate dose-response curve. Therefore, IC_{50} values can change regarding the fit of the dose-response curve and the functional model used. This makes it even more difficult to reliably compare cytotoxicity data, as different research groups may choose different curve fits and different quality parameters for these estimations.

It was not possible to distinguish between antiproliferative and cytotoxic effects in the algae, which showed only weak effects. It is possible that growth was inhibited in these samples due to antiproliferative effects, which can not be differentiated from a small amount of cells that died due to cytotoxic effects. It is conceivable that the effects observed in the algae, for which only estimated values are given, are not cytotoxic. Instead, they could be the results of antiproliferative activities.

As our study is the first toxicity screening of the chosen species comparisons to other studies had to be made with some caveats. As no studies on the same species were available, studies dealing with species of the same genus were chosen for comparison. However, it should be noted that species of the same genus may have different cytotoxic activities, which can be a reason for different results, e. g. different culture conditions as well as different solvents and extraction methods. Depending on the polarity of the solvents, the extracted substances and their cytotoxicity can vary. For example, phenolic substances are best extracted with methanol and ethanol, tannins are best extracted with water and xanthones and are best extracted with non-polar substances such as hexane [57, 58]. We chose solvents that cover a broad range of polarities to extract as many different components as possible. This is in line with our screening approach, which aims to get an overview of the cytotoxic potentials rather than performing an in-depth analysis of a single substance. We also chose solvents that did not affect the growth of the cell line at the concentrations tested. Other studies have shown that substances can exhibit stronger cytotoxic effects if they are used in solvents with higher cytotoxic potential [59].

In future experiments, it might be beneficial to include solvents such as DMSO, which is a universal amphiphilic solvent [59]. The exposure time and the cell lines used also varied, which impacted the results as well. We chose widely used and recognized cell lines and exposure times appropriate for these cell lines to ensure that the cells were still viable at the end of the exposure.

Of the eight microalgae tested, the highest cytotoxicity potential in our study was calculated for the ethanolic and acetonic extracts of Stichococcus sp. in both cell lines. The in vitro toxicity of this genus was also measured by Gürlek et al. (2020), who extracted Stichococcus (S.) bacillaris with methanol and examined the cytotoxic effect on HEP G2 cells after 48 hours of exposure, and Atasever-Arslan et al. (2016), who investigated the effect of methanolic S. bacillaris extract on human leukemia and endothelial cell lines [60, 61]. Both report their results in mg extract ml-1, which explains the perceived differences as it is not known what amount of extracted algae corresponds to their results. In the study by Gürlek et al. (2020), the IC_{50} was set at 0.37 ± 0.09 mg extract ml^{-1} and described as non-toxic, because significant toxic effects were defined as an IC_{50} below 0.02 mg ml^{-1} [61, 56]. The results in our study were 0.18 mg extracted biomass ml^{-1} and 0.52 mg extracted biomass ml^{-1} in acetone and ethanol extracts, respectively. In the study by Atasever-Arslan et al. (2016), S. bacillaris had no cytotoxic effect on the non-cancerous endothelial cell line at concentrations up to 0.5 mg extract ml^{-1} and cell viability of both leukaemia cell lines was reduced by 50 % at 0.05 and 0.5 mg ml^{-1}, respectively [60]. This demonstrates the varying effects that algae extracts can have on different cell lines. Despite the differences in solvents, calculation and different subspecies of microalgae, the components that led to the cytotoxic effect in the investigation by Gürlek et al. (2020) and Atasever-Arslan et al. (2016) may be the same components that cause cytotoxicity in both cell lines in our study [60, 61]. In the study by Atasever-Arslan et al. (2016), these components were further investigated and it was demonstrated that extracts of S. bacillaris lead to the induction of apoptotic pathways. Potential molecules that regulate this mechanism could be 2,2,4-trimethyl-1,3-pentanediyl bis (2-methylpropanoate), 5,6-dihydroergosterol and 9-octadecenamide, which were present in S. bacillaris extracts and the essential oil generated from S. bacillaris. These effects could only be induced in leukemia cells in the study by Atasever-Arslan et al. (2016), but they may show the same effect on our cell lines and are extracted by ethanol (as another short-chained alcohol) as well [60]. Further studies are required to investigate this further and to verify whether the cytotoxic effects are exclusive to cancerous cells.

In our study, M. bisecta showed cytotoxicity in both cell lines tested. The cytotoxicity of M. bisecta has not been studied so far, but its antibacterial effect on methanolic extracts has been described by Santhakumaran et al. (2020) [62]. It is possible that bioactive compounds that lead to an antibacterial effect also affect cell lines. For example, free fatty acids, which are also found in microalgae such as M. bisecta, have antimicrobial effects due to their effect on the cell wall, which can lead to the dissolution of the membrane [63]. At lower concentrations, they can disrupt the electron transport chain and the oxidative phosphorylation, impair nutrient uptake, and inhibit enzyme activities, among other mechanisms of action [63]. In cell lines, free fatty acids have been shown to have cytotoxic and anticancer effects by triggering cell cycle arrest and inducing apoptosis of tumor cells [64]. Even though the mechanisms of action of free fatty acids against microorganisms differ from those that lead to cytotoxicity, it could be possible that free fatty acids lead to the observations in the ethanol and acetone extracts in our study as well as to the antibacterial effects documented by Santhakumaran et al. (2020) [62].

T. obliquus displayed cytotoxicity of ethanol and acetone extracts in CHO-k1 cells and cytotoxicity of ethanol extracts on HEP G2. No cytotoxic effect of hexane and aqueous extracts was detected. The cytotoxicity of this microalgae has been extensively studied and several publications have been published on the presence of bioactive compounds and cytotoxicity towards different cell lines. In these studies, different strains of Tetradesmus, different cell lines, solvents and incubation times were tested, which explains the wide range of results. Marrez et al. (2019) tested the cytotoxicity of Scenedesmus (S.) obliquus (synonym for T. obliquus [14]) in diethyl ether extracts in HEP G2 and two other cancer cell lines as well as in three non-cancer cell lines after 48 hours of exposure at concentrations up to 0.18 mg extract ml^{-1} [65]. The IC_{50} of this extract in HEP G2 was 0.04 mg extract ml^{-1} and no cytotoxicity against non-cancerous cell lines could be detected [65]. Marrez et al. (2019) investigated potential molecules responsible for the cytotoxicity and found that 3-hexadecyloxycarbonyl-5-(2-hydroxyl)-4-methylimidazolium was present in the extracts. The substance has shown cytotoxic effects in other studies [65, 66]. In contrast to the study by Marrez et al. (2019), no selective toxicity towards cancer cells was detected in our experiment [65]. The fatty acid fraction of S. obliquus was investigated in more detail by Abd el Baky et al. (2014) and an IC_{50} of 0.01 -0.02 mg oil ml^{-1} was shown for S. obliquus oil in the cell lines HEP G2, HCT116 and MCF7 after 48 hours of exposure [67]. It is discussed that fatty acids, carotenoids and phenolic compounds can induce apoptosis in cancer cell lines and could be the cause of the antiproliferative effect [67]. Singab et al. (2018) showed the antiproliferative effect of polysaccharides extracted from Scenedesmus obliquus [68]. The IC_{50} of these polysaccharides on HEP G2 cells was estimated to be 0.1 mg polysaccharides ml^{-1}, indicating that polysaccharides could be another potential substance leading to cytotoxicity. Furthermore, in various studies the effect of different extracts of Scenedesmus sp. on tumoral- and non-tumoral cell lines [69-72] was tested. In contrast to the results of Reyna-Martinez et al. (2018) and Zaharieva et al. (2022), Ördög et al. demonstrated that non-

cancerous cell lines can also be inhibited by sufficiently high concentrations of Scenedesmus sp. extracts [70-72]. In conclusion, the different studies conducted on Scenedesmus sp. indicate that different substances present in the microalgae, including fatty acids, carotenoids, polysaccharides and phenolic compounds, but also more specific substances such as 3-hexadecyloxycarbonyl-5-(2-hydroxyl)-4-methylimidazolium, can be responsible for the cytotoxicity observed in our study.

In our experiment, cytotoxicity of the ethanol and acetone extracts of S. minor was observed against both cell lines, although this was more pronounced in the acetone extract. Comparable studies on this microalga are currently not available. The antibacterial effects of the aqueous extracts of two Spongiochloris sp. Strains were investigated by Ördög et al. (2004) but were less distinct than those of the other microalgae extracts tested and were therefore not quantified further [72]. Since Spongiochloris spp. are rich in polyphenolic compounds and these are known for their biological activities, these may explain both the antimicrobial effect in the study by Ördög et al. (2004) and the cytotoxic effect on the cells tested [72, 73].

The microalgae species M. salina was formerly known as Nannochloropsis (N.) salina [14]. In our study, ethanol extracts of M. salina showed a cytotoxic effect against both tested cell lines with an IC_{50} of 0.67 mg extracted biomass ml^{-1} in CHO-k1 cells and 0.87 mg extracted biomass ml^{-1} in HEP G2 cells, while acetone extracts were only cytotoxic against HEP G2 cells with an IC_{50} of 7.31 mg extracted biomass ml^{-1}. Other research groups tested N. oculate, N. oceanica or undefined Nannochloropsis sp. for their cytotoxic potential and safety in vivo and in vitro [50, 74-76, 77, 78]. In the study by Sanjeewa et al. (2016), N. oculata was first extracted with methanol and the resulting extract was fractionated using a hexane-ethyl acetate step-gradient elution [78]. None of the extracts obtained had cytotoxic effects on Vero cells at concentrations up to 0.03 mg fractionated extract ml^{-1}, but HL-60 cells were inhibited by the less polar extracts. Ávila-Roman et al. (2016) tested oxylipin derived from N. gaditana on HT-29 and UACC-62 cells to monitor the effects on cancer and non-cancer cell lines [74]. While no IC_{50} could be determined in the non-cancerous cell line (HT-29), an IC_{50} of up to 53 µM oxylipin was found in UACC-62 cells [74]. Venkatraman et al (2022) examined the effect of methanolic extracts of Nannochloropsis sp. on HEP G2 cells, which resulted in an IC_{50} of 0.4 mg extract ml^{-1} after 24 hours [77]. None of these studies can be quantitatively compared to our studies as they only investigated specific fractions of Nannochloropsis sp. extracts and calculated their IC_{50} based on this fraction [78, 74] or they calculated the IC_{50} based on the extract concentration [77]. Niccolai et al. (2017) proved the cytotoxic effect of aqueous extracts of N. oceanica in vitro on fibroblasts with an IC_{50} of 11.2 mg extracted biomass ml^{-1}, which could not be replicated in our study. However, we found cytotoxic effects of M. salina on HEP G2 cells. These could be caused, for example, by the oxylipin or the same fractions extracted by Sanjeewa et al. (2016). In contrast to the previously discussed microalgae, there are in vivo studies on N. oculate. Kagan and Matulka (2015) demonstrated that oral administration of N. oculata solution at a concentration of 1 × 10E8 algal cells ml^{-1} at a dose of 10 ml kg^{-1} for 14 days did not cause toxic effects in rats [75]. In another study, Kagan et al. (2014) tested the safety of an oil produced from N. oculata using CHO cells for genotoxicity assessments in vitro and an in vivo assessment of toxicity in rats [79]. The IC_{50} for the algal oil used to determine chromosome aberration ranged from 0.004 mg Almega PL ml^{-1} to 0.008 mg Almega PL ml^{-1} after 21 hours of exposure and no genotoxicity was observed in the chromosome aberration test, while the dose of 2000 mg kg^{-1} per day showed no toxic effects in rats [79]. These findings prove that even an IC_{50} of less than 0.01 mg ml^{-1} does not necessarily correspond to in vivo toxicity. Furthermore, Neumann et al. (2018) demonstrated that the total biomass of N. oceanica had no toxic effects on mice in vivo [76, 50]. This is further evidence that the in vitro effects of algal extracts may not correlate with in vivo toxicity. This is often the case in in vitro cytotoxicity studies, as the effect of digestion on bioactive substances is not replicated in vitro. Furthermore, cell cytotoxicity assays cannot predict whether bioactive substances are resorbed in vivo at all. The in vivo studies indicate that the use of M. salina in human diet is possible despite its cytotoxic effects. Nevertheless, in vivo studies with exactly these algae are needed, as even closely related algae may differ in their toxicity.

In B. braunii, the ethanolic extract inhibited both cell lines. The water extract only inhibited CHO-k1 cells, while the hexane extract only affected HEP G2 cells. Hexane, diethyl ether, acetone and water extracts of Botryococcus sp. were investigated by Custódio et al. (2015) [80]. They found that cytotoxic effects on neuroblastoma cells (SH-SY5Y) started at a concentration of 0.05 mg ml^{-1} of B. braunii extracts, but it was not specified which extract led to these effects [80]. These cytotoxic effects are about ten times stronger than the cytotoxicity measured in our investigations, which started at an IC_{50} of 0.57 mg extracted biomass ml^{-1}. This could be consistent with our results if the extract used by Custódio et al. (2015) corresponds to about 10 % of the weight of the dried biomass [80]. In addition, the anticancer effects of ethanolic extracts of B. braunii on three cancer- and one non-cancer cell line were investigated by İnan Benan et al. (2021) [81]. An IC_{50} could not be determined, as B. braunii only had a weak cytotoxic effect on one cell line (SHSY-5Y cancer cells) after 24 hours and 80 % of the cells in this cell line were still viable even at the highest concentration (0.38 mg oil ml^{-1}). This differs from our results as the ethanolic extract had an IC_{50} of 0.57 mg extracted biomass ml^{-1} against the HEPG2 cancer cell line. The stationary phase of B. braunii has a total lipid content of 51 g in 100 g of dried microalgae [34]. Therefore, 0.57 mg B. braunii could correspond to a maximum of 0.29 mg extracted oil. The difference between

our results and those of İnan Benan et al. (2021) could be due to the difference in the cell lines used or the shorter exposure [81].

In C. novae-angliae, only the ethanolic extract had cytotoxic effects on both cell lines. The antigenotoxic effects of C. humicola carotenoids were described by Bhagavathy and Sumathi (2012) in human lymphocytes, where concentrations of 0.1 mg ml^{-1} - 0.3 mg carotenoids ml^{-1} protected the lymphocytes from genotoxic effects [82]. In addition, no cytotoxicity could be detected at these concentrations [82]. Since the IC_{50} values determined for C. novae-angliae in the present study were 1.76 mg extracted biomass ml^{-1} and 4.6 mg extracted biomass ml^{-1} in HEP G2 and CHO-k1 cells, respectively, the study by Bhagavathy and Sumathi (2012) may indicate that this cytotoxic effect is not caused by carotenoids but by another extracted component. The compounds mentioned in the section of other algae, e. g. polysaccharides, fatty acids or phenolic components, are possible candidates [82].

In our study, T. suecica did not show an IC50 below 15 mg extracted biomass ml^{-1} for any of the extracts tested. T. suecica is the only species in our study that belongs to a genus in which one species is approved for use in human nutrition. T. chuii has been approved as a novel food in the EU [83]. It was tested in vivo at doses up to 2500 mg lyophilized biomass kg^{-1} per day in a 90-day feeding study in rats and showed no signs of toxicity [84]. The safety of another strain of Tetraselmis sp. was discussed by Custódio et al. (2014) [69]. Different extracts were tested on HEP G2 and S17 cells, resulting in an IC_{50} of 0.06 mg extract ml^{-1} for hexane extracts on HEP G2 cells and a similar IC_{50} on S17 cells after 72 hours of exposure [69]. It is not possible to compare these findings with our study as it is unknown how much dried hexane extract correlates to our amount of extracted biomass. Rosa et al. (2005) investigated the effect of T. suecica on VERO, MDCK, and Hela cells [85]. The maximum nontoxic dose of lyophilized T. suecica was 0.05 mg lyophilized algae ml^{-1} on VERO and MDCK cells after 48 hours. For the extracts, the cytotoxicity depended on the solvent, and for methanol, chloroform, and hexane, the maximum nontoxic dose was 0.02, 0.04, and 0.25 mg extract ml^{-1} on Hela cells after 48 hours of exposure, respectively. The results of Rosa et al. (2005) are in agreement with those of Custódio et al. (2014) but are in contrast to our research, as our extracts out of 0.50 mg lyophilized algae did not show cytotoxicity [69, 85]. Since the cytotoxicity of methanol extracts was highest in the study by Rosa et al. (2005), it is possible that the cytotoxicity of the lyophilized algae was caused by a component that was not extracted through our solvents, even though most substances extracted with methanol should be extracted with ethanol as well [85]. Parra-Riofrío et al. (2020) examined the effect of exopolysaccharides from T. suecica on cancer cells and found that the cytotoxic effect depended on whether T. suecica was grown as a total or acid autotrophic or heterotrophic organism [17]. The exopolysaccharides from total autotrophic and heterotrophic cultures reached their IC_{50} at about 10-fold higher concentrations than those from acid autotrophic cultures. The results are not comparable to our study, as the IC_{50} is displayed in mg exopolysaccharides ml^{-1}, but the study by Parra-Riofrío et al. (2020) demonstrated another problem when comparing cytotoxicity results, as these are highly dependent on the way the microalgae are grown [17]. In conclusion, the results of our study suggest that T. suecica may be suitable for human nutrition, as no cytotoxicity could be detected. However, it must be noted that cytotoxic effects of Tetraselmis sp. have been observed in other studies. It is unclear whether these are related to in vivo toxicity, as T. chuii has been extensively tested in vivo.

In general, further investigation of the generated data is required. This could be done specifically in relation to the cytotoxic compound by analyzing the cytotoxic extracts, identifying potentially cytotoxic molecules and investigating the specific metabolic pathways by proteomic or metabolomic approaches. With regard to the use of microalgae in human nutrition, it would be important to check whether in vitro cytotoxicity corresponds to in vivo cytotoxicity. To check this, an assay with Artemia salina could be suitable. It would also be necessary to conduct genotoxicity tests. The IC_{50} values we determined could be a starting point for these further studies.

Conclusion

Microalgae play a growing role in human nutrition and the microalgae investigated could enrich the human diet with different nutrients, some of which are otherwise only present in particular food categories. Our study showed that the cytotoxicity of most algae was weaker than previously described. This might result from different culture conditions and strains. Additionally, most of the studies discussed were conducted on other cell lines, which makes a direct comparison difficult.

Only T. suecica showed no cytotoxic effects in any of the extracts tested. However, it is also possible that the cytotoxic effects in our study are due to compounds in the microalgae that are non-toxic or even beneficial to humans. There are studies demonstrating that free fatty acids can cause cytotoxicity in HEP G2 and CHO-k1 cells, which may have been the cause of the measured cytotoxic effects in our study [86, 87]. Therefore, further studies are needed to determine whether the cytotoxic effects shown also have correlating effects in vivo. Assays relaying on invertebrates such as Artemia salina could be an intermediate step before continuing with in vivo tests on vertebrates. Microalgae such as T. suecica and M. salina would be suitable candidates for these examinations as they provide valuable nutrients and there is already data on other microalgae of this genus generated from in vivo examinations.

On the other hand, microalgae with a selective cytotoxic effect on HEP G2 could also show an anticancer activity on

other tumor cell lines or even in vivo. In our study, the only cell line exhibited selective cytotoxic effects on HEP G2 cells was C. novae-angliae. The cytotoxicity of the ethanol extract resulted in an IC_{75} of 1.98 mg extracted biomass ml^{-1} for HEP G2 cells, while the IC_{75} for CHO-k1 cells was more than 15 mg ml^{-1}. Although selective cytotoxicity was demonstrated, the effects may be too weak to be useful for cancer research. It is possible that these effects could be stronger when more specific algal metabolites are tested. Further studies are required to isolate the algal metabolites that induce anticancer effects, determine their structures and verify the effects on other cell lines.

Conflict of interest

The authors declare that there is no conflict of interest.

Authors' contribution

The research project was carried out at the Institute of Food Hygiene of the University of Leipzig under the supervision of Prof. Dr. Peggy Braun and Dr. Claudia Wiacek. The experimental concept was developed by Tomke Prüser with the above-mentioned persons and Prof. Dr. Carola Griehl. Prof. Dr. Carola Griehl selected the microalgae species and performed the experimental design for the production of microalgae biomass at the Anhalt University of Applied Sciences. The experiments were carried out, evaluated and statistically analyzed by Tomke Prüser at the Institute of Food Hygiene of the University of Leipzig. The manuscript was written by Tomke Prüser and it was critically reviewed in collaboration with Prof. Dr. Peggy Braun, Prof. Dr. Carola Griehl and Dr. Claudia Wiacek.

Acknowledgement

We thank S. Berft for her excellent technical assistance.

Funding

This research was funded by the Federal Ministry of Education and Research (NovAL project) grant numbers 031B0366C and 031B0366D.

Supplementary material

The supplementary material (Table S1) is available at: https://file.luminescience.cn/FNDS-297%20Supplementary%20Material.pdf.

References

[1] Borowitzka MA. Systematics, taxonomy and species names: Do they matter? In: Borowitzka MA, Beardall J, Raven JA (eds.) *The physiology of microalgae*. Springer International Publishing; 2016. p. 665-81.

[2] Guiry MD. How many Species of Algae are there? *Journal of Phycology*. 2012; 48(5):1057-63. Available from: doi:10.1111/j.1529-8817.2012.01222.x.

[3] Algae Products Market - Global Industry Size, Share, Trends, Algae Products Market - Global Industry Size, Share, Trends, Opportunity, and Forecast, 2018-2028F. ID: 4790320.; 2023. Available from: https://www.researchandmarkets.com/reports/4790320/algae-products-market-global-industry-size#rela1-4521311. [Accessed 11th October 2024].

[4] Borowitzka MA. High-value products from microalgae—their development and commercialisation. *Journal of Applied Phycology*. 2013; 25(3): 743-56. doi:10.1007/s10811-013-9983-9.

[5] Pulz O, Gross W. Valuable products from biotechnology of microalgae. *Applied Microbiology and Biotechnology*. 2004; 65(6): 635-48. doi:10.1007/s00253-004-1647-x.

[6] Safafar H, van Wagenen J, Møller P, Jacobsen C. Carotenoids, Phenolic Compounds and Tocopherols Contribute to the Antioxidative Properties of Some Microalgae Species Grown on Industrial Wastewater. *Marine Drugs*. 2015; 13(12): 7339-56. doi:10.3390/md13127069.

[7] Sandgruber F, Höger A-L, Kunze J, Schenz B, Griehl C, Kiehntopf M et al. Impact of Regular Intake of Microalgae on Nutrient Supply and Cardiovascular Risk Factors: Results from the NovAL Intervention Study. *Nutrients*. 2023; 15(7). doi:10.3390/nu15071645.

[8] Food and Agriculture Organization of the United Nations (FAO). Global Fishery and Aquaculture Production Statistics. Available from: www.fao.org/fishery/statistics/software/fishstatj. [Accessed 11th October 2024].

[9] Enzing C, Ploeg M, Barbosa M, Sijtsma L. *Microalgae-based products for the food and feed sector: An outlook for Europe*. Luxembourg: Publications Office; 2014. (EUR, Scientific and technical research series; vol 26255).

[10] Prüser TF, Braun Peggy Gabriele, Wiacek C. Microalgae as a novel food: Potential and legal framework. *Ernährungsumschau*. 2021; 68(4): 78-85. doi:10.4455/eu.2021.016.

[11] Del Mondo A, Smerilli A, Sané E, Sansone C, Brunet C. Challenging microalgal vitamins for human health. *Microbial Cell Factories*. 2020; 19(1): 201. doi:10.1186/s12934-020-01459-1.

[12] García JL, Vicente M de, Galán B. Microalgae, old sustainable food and fashion nutraceuticals.

Microbial Biotechnology. 2017; 10(5): 1017-24. doi:10.1111/1751-7915.12800.

[13] Rhodes L, Wood S. Micro-algal and Cyanobacterial Producers of Biotoxins. In: Rossini GP (ed.) *Toxins and biologically active compounds from microalgae Origin, chemistry and detection*. Boca Raton, FL: CRC Press Taylor & Francis Group; 2014. p. 21-51

[14] Guiry, M.D. & Guiry, G.M. AlgaeBase; 2019. Available from: http://www.algaebase.org. [Accessed 11th October 2024].

[15] Tasker RA. Chemistry and Detection of Domoic Acid and Isomers. In: Rossini GP (ed.) *Toxins and biologically active compounds from microalgae Origin, chemistry and detection*. Boca Raton, FL: CRC Press Taylor & Francis Group; 2014. p. 232-50

[16] Jiang Y, Xie P, Chen J, Liang G. Detection of the hepatotoxic microcystins in 36 kinds of cyanobacteria Spirulina food products in China. *Food Additives and Contaminants. Part A, Chemistry, Analysis, Control, Exposure & Risk Assessment*. 2008; 25(7): 885-94. doi:10.1080/02652030701822045.

[17] Parra-Riofrío G, García-Márquez J, Casas-Arrojo V, Uribe-Tapia E, Abdala-Díaz RT. Antioxidant and Cytotoxic Effects on Tumor Cells of Exopolysaccharides From Tetraselmis suecica (Kylin) Butcher Grown Under Autotrophic and Heterotrophic Conditions. *Marine Drugs*. 2020; 18(11). doi:10.3390/md18110534.

[18] Rellán S, Osswald J, Saker M, Gago-Martinez A, Vasconcelos V. First detection of anatoxin-a in human and animal dietary supplements containing cyanobacteria. *Food and Chemical Toxicology*. 2009; 47(9): 2189-95. doi:10.1016/j.fct.2009.06.004.

[19] Sánchez-Parra E, Boutarfa S, Aboal M. Are Cyanotoxins the Only Toxic Compound Potentially Present in Microalgae Supplements? Results from a Study of Ecological and Non-Ecological Products. *Toxins (Basel)*. 2020; 12(9). doi:10.3390/toxins12090552.

[20] Yu F-Y, Liu B-H, Chou H-N, Chu FS. Development of a sensitive ELISA for the determination of microcystins in algae. *Journal of Agricultural and Food Chemistry*. 2002; 50(15): 4176-82. doi:10.1021/jf0202483.

[21] Leflaive J, Ten-Hage L. Algal and cyanobacterial secondary metabolites in freshwaters: a comparison of allelopathic compounds and toxins. *Freshwater Biology*. 2007; 52(2): 199-214. doi:10.1111/j.1365-2427.2006.01689.x.

[22] EFSA Panel on Dietetic Products, Nutrition and Allergies. Guidance on the preparation and presentation of an application for authorisation of a novel food in the context of Regulation (EU) 2015/2283. *EFS2*. 2016; 14(11): 54. doi:10.2903/j.efsa.2016.4594.

[23] Cabanelas ITD, Marques SSI, Souza CO de, Druzian JI, Nascimento IA. Botryococcus what to do with it? Effect of nutrient concentration on biorefinery potential. *Algal Research*. 2015; 11:43-9. doi:10.1016/j.algal.2015.05.009.

[24] Lang I, Hodac L, Friedl T, Feussner I. Fatty acid profiles and their distribution patterns in microalgae: a comprehensive analysis of more than 2000 strains from the SAG culture collection. *BMC Plant Biology*. 2011; 11:124. doi:10.1186/1471-2229-11-124.

[25] Makri A, Bellou S, Birkou M, Papatrehas K, Dolapsakis NP, Bokas D et al. Lipid synthesized by micro-algae grown in laboratory- and industrial-scale bioreactors. *Engineering in Life Sciences*. 2011; 11(1): 52-8. doi:10.1002/elsc.201000086.

[26] Patil V, Källqvist T, Olsen E, Vogt G, Gislerød HR. Fatty acid composition of 12 microalgae for possible use in aquaculture feed. *Aquaculture International*. 2007; 15(1): 1-9. doi:10.1007/s10499-006-9060-3.

[27] Santos-Sánchez NF, Valadez-Blanco R, Hernández-Carlos B, Torres-Ariño A, Guadarrama-Mendoza PC, Salas-Coronado R. Lipids rich in ω-3 polyunsaturated fatty acids from microalgae. *Applied Microbiology and Biotechnology*. 2016; 100(20): 8667-84. doi:10.1007/s00253-016-7818-8.

[28] Zanella L, Vianello F. Microalgae of the genus Nannochloropsis: Chemical composition and functional implications for human nutrition. *Journal of Functional Foods*. 2020; 68:103919. doi:10.1016/j.jff.2020.103919.

[29] Sandgruber F, Gielsdorf A, Baur AC, Schenz B, Müller SM, Schwerdtle T et al. Variability in Macro- and Micronutrients of 15 Commercially Available Microalgae Powders. *Marine Drugs*. 2021; 19(6). doi:10.3390/md19060310.

[30] Brown MR, Mular M, Miller I, Farmer C, Trenerry C. The vitamin content of microalgae used in aquaculture. *Journal of Applied Phycology*. 1999; 11(3): 247-55. doi:10.1023/A:1008075903578.

[31] Edelmann M, Aalto S, Chamlagain B, Kariluoto S, Piironen V. Riboflavin, niacin, folate and vitamin B12 in commercial microalgae powders. *Journal of Food Composition and Analysis*. 2019; 82:103226. doi:10.1016/j.jfca.2019.05.009.

[32] Da Silva MET, Correa KdP, Martins MA, Da Matta SLP, Martino HSD, Coimbra JSdR. Food safety, hypolipidemic and hypoglycemic activities, and in vivo protein quality of microalga Scenedesmus obliquus in Wistar rats. *Journal of Functional Foods*. 2020; 65:103711. doi:10.1016/j.jff.2019.103711.

[33] Morowvat MH, Ghasemi Y. Screening of some Naturally Isolated Microalgal Strains for Polyunsaturated Fatty Acids Production. *Asian Journal of Pharmaceutical Research and Health Care*. 2016; 8(4): 122. doi:10.18311/ajprhc/2016/6113.

[34] Sandgruber F, Gielsdorf A, Schenz B, Müller SM, Schwerdtle T, Lorkowski S et al. Variability in Macro- and Micronutrients of 15 Rarely Researched Microalgae. *Marine Drugs*. 2023; 21(6). doi:10.3390/md21060355.

[35] Klejdus B, Kopecký J, Benesová L, Vacek J. Solid-phase/supercritical-fluid extraction for liquid chromatography of phenolic compounds in freshwater microalgae and selected cyanobacterial species. *Journal of Chromatography A*. 2009; 1216(5): 763-71. doi:10.1016/j.chroma.2008.11.096.

[36] Bravo L. Polyphenols: chemistry, dietary sources, metabolism, and nutritional significance. *Nutrition Reviews*. 1998; 56(11): 317-33. doi:10.1111/j.1753-4887.1998.tb01670.x.

[37] Morais MG de, Da Vaz BS, Morais EG de, Costa JAV. Biologically Active Metabolites Synthesized by Microalgae. *BioMed Research International*. 2015; 2015: 835761. doi:10.1155/2015/835761.

[38] Putnam K, Bombick D, Avalos J, Doolittle D. Comparison of the Cytotoxic and Mutagenic Potential of Liquid Smoke Food Flavourings, Cigarette Smoke Condensate and Wood Smoke Condensate. *Food and Chemical Toxicology*. 1999; 37(11): 1113-8. doi:10.1016/S0278-6915(99)00104-0.

[39] Tofiño-Rivera A, Ortega-Cuadros M, Galvis-Pareja D, Jiménez-Rios H, Merini LJ, Martínez-Pabón MC. Effect of Lippia alba and Cymbopogon citratus essential oils on biofilms of Streptococcus mutans and cytotoxicity in CHO cells. *Journal of Ethnopharmacology*. 2016; 194: 749-54. doi: 10.1016/j.jep.2016.10.044.

[40] Whitwell J, Fowler P, Allars S, Jenner K, Lloyd M, Wood D et al. 2-Aminoanthracene, 5-fluorouracil, colchicine, benzoapyrene, cadmium chloride and cytosine arabinoside tested in the in vitro mammalian cell micronucleus test (MNvit) in Chinese hamster ovary (CHO) cells at Covance Laboratories, Harrogate UK in support of OECD draft Test Guideline 487. *Mutation Research*. 2010; 702(2): 237-47. doi:10.1016/j.mrgentox.2010.05.004.

[41] Organisation for Economic Co-operation and Development. *OECD Guidelines for the Testing of Chemicals 487: In vitro mammalian cell micronucleus test*. Paris: OECD Publishing; 2023. (OECD Guidelines for the Testing of Chemicals. Section 4, Health Effects).

[42] EFSA Scientific Committee. Scientific opinion on genotoxicity testing strategies applicable to food and feed safety assessment. *EFSA Journal*. 2011; 9(9). doi:10.2903/j.efsa.2011.2379.

[43] Liu ZH, Zeng S. Cytotoxicity of ginkgolic acid in HepG2 cells and primary rat hepatocytes. *Toxicology Letters*. 2009; 187(3): 13106. doi: 10.1016/j.toxlet.2009.02.012.

[44] Machana S, Weerapreeyakul N, Barusrux S, Nonpunya A, Sripanidkulchai B, Thitimetharoch T. Cytotoxic and apoptotic effects of six herbal plants against the human hepatocarcinoma (HepG2) cell line. *Chinese Medicine*. 2011; 6(1): 39. doi:10.1186/1749-8546-6-39.

[45] Senthilraja P, Kathiresan K. In vitro cytotoxicity MTT assay in Vero, HepG2 and MCF -7 cell lines study of Marine Yeast. *Journal of Applied Pharmaceutical Science*. 2015:80-4. doi:10.7324/JAPS.2015.50313.

[46] Salem N, Eggersdorfer M. Is the world supply of omega-3 fatty acids adequate for optimal human nutrition? *Current Opinion in Clinical Nutrition & Metabolic Care*. 2015; 18(2):147-54. doi:10.1097/MCO.0000000000000145.

[47] Calder PC. Very long-chain n-3 fatty acids and human health: fact, fiction and the future. *Proceedings of the Nutrition Society*. 2018; 77(1): 52-72. doi:10.1017/S0029665117003950.

[48] Schade S, Stangl GI, Meier T. Distinct microalgae species for food—part 2: comparative life cycle assessment of microalgae and fish for eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and protein. *Journal of Applied Phycology*. 2020; 32(5): 2997-3013. doi:10.1007/s10811-020-02181-6.

[49] Himuro S, Ueno S, Noguchi N, Uchikawa T, Kanno T, Yasutake A. Safety evaluation of Chlorella sorokiniana strain CK-22 based on an in vitro cytotoxicity assay and a 13-week subchronic toxicity trial in rats. *Food and Chemical Toxicology*. 2017; 106(Pt A): 1-7. doi:10.1016/j.fct.2017.05.025.

[50] Niccolai A, Bigagli E, Biondi N, Rodolfi L, Cinci L, Luceri C et al. In vitro toxicity of microalgal and cyanobacterial strains of interest as food source. *Journal of Applied Phycology*. 2017; 29(1): 199-209. doi:10.1007/s10811-016-0924-2.

[51] Bechelli J, Coppage M, Rosell K, Liesveld J. Cytotoxicity of algae extracts on normal and malignant cells. *Leukemia Research and Treatment*. 2011: 373519. doi:10.4061/2011/373519.

[52] Balaji M, Dhanapal T, Chidambara Vinayagam S, S Balakumar B. Anticancer, Antioxidant Activity and GC-MS Analysis of selected Microalgal Members of Chlorophyceae. *International Journal of Pharmaceutical Sciences and Research*. 2017; 8(8): 3302-14. doi:10.13040/IJPSR.0975-8232.8(8).3302-14.

[53] Daniel R. Antibacterial Activity of the Marine Diatom Skeletonema costatum Against Selected Human Pathogens. *International Journal of Current Pharmaceutical Review and Research*. 2016; 7(5): 233-6. Available from: https://impactfactor.org/PDF/IJCPR/7/IJCPR,Vol7,Issue5,Article1.pdf. [Accessed 11th October 2024].

[54] Gille A, Trautmann A, Gomez MR, Bischoff SC, Posten C, Briviba K. Photoautotrophically Grown Chlorella vulgaris Shows Genotoxic Potential but No Apoptotic Effect in Epithelial Cells. *Journal of Agricultural and Food Chemistry*. 2019; 67(31): 8668-76. doi:10.1021/acs.jafc.9b03457.

[55] Boyd MR. The NCI In Vitro *Anticancer Drug Discovery Screen. In: Teicher BA (ed.) Anticancer Drug Development Guide*. Totowa, NJ: Humana Press; 1997. p. 23-42.

[56] Tan ML, Sulaiman SF, Najimuddin N, Samian MR, Muhammad TST. Methanolic extract of Pereskia bleo (Kunth) DC. (Cactaceae) induces apoptosis in breast carcinoma, T47-D cell line. *Journal of Ethnopharmacology*. 2005; 96(1-2):287-94. doi:10.1016/j.jep.2004.09.025.

[57] Ngawhirunpat T, Opanasopi P, Sukma M, Sittisombut C, Kat A, Adachi I. Antioxidant, free radical-scavenging activity and cytotoxicity of different solvent extracts and their phenolic constituents from the fruit hull of mangosteen (Garcinia mangostana). *Pharmaceutical Biology*. 2010; 48(1): 55-62. doi:10.3109/13880200903046138.

[58] Yusoff IM, Mat Taher Z, Rahmat Z, Chua LS. A review of ultrasound-assisted extraction for plant bioactive compounds: Phenolics, flavonoids, thymols, saponins and proteins. *Food Research International*. 2022; 157: 111268. doi:10.1016/j.foodres.2022.111268.

[59] Ilieva Y, Dimitrova L, Zaharieva MM, Kaleva M, Alov P, Tsakovska I et al. Cytotoxicity and Microbicidal Activity of Commonly Used Organic Solvents: A Comparative Study and Application to a Standardized Extract from Vaccinium macrocarpon. *Toxics*. 2021; 9(5). doi:10.3390/toxics9050092.

[60] Atasever-Arslan B, Yilancioglu K, Kalkan Z, Timucin AC, Gür H, Isik FB et al. Screening of new antileukemic agents from essential oils of algae extracts and computational modeling of their interactions with intracellular signaling nodes. *European Journal of Pharmaceutical Sciences*. 2016; 83:120-31. doi:10.1016/j.ejps.2015.12.001.

[61] Gürlek C, Yarkent Ç, Köse A, Tuğcu B, Gebeloğlu IK, Öncel SŞ et al. Screening of antioxidant and cytotoxic activities of several microalgal extracts with pharmaceutical potential. *Health and Technology*. 2020; 10(1): 111-7. doi:10.1007/s12553-019-00388-3.

[62] Santhakumaran P, Ayyappan SM, Ray JG. Nutraceutical applications of twenty-five species of rapid-growing green-microalgae as indicated by their antibacterial, antioxidant and mineral content. *Algal Research*. 2020; 47:101878. doi:10.1016/j.algal.2020.101878.

[63] Desbois AP, Smith VJ. Antibacterial free fatty acids: activities, mechanisms of action and biotechnological potential. *Applied Microbiology and Biotechnology*. 2010; 85(6): 1629-42. doi:10.1007/s00253-009-2355-3.

[64] Jóźwiak M, Filipowska A, Fiorino F, Struga M. Anticancer activities of fatty acids and their heterocyclic derivatives. *European Journal of Pharmacology*. 2020; 871: 172937. doi:10.1016/j.ejphar.2020.172937.

[65] Marrez DA, Naguib MM, Sultan YY, Higazy AM. Antimicrobial and anticancer activities of Scenedesmus obliquus metabolites. *Heliyon*. 2019; 5(3): e01404. doi:10.1016/j.heliyon.2019.e01404.

[66] Suriyavathana M., Indupriya S. GC-MS analysis of phytoconstituents and concurrent determination of flavonoids by HPLC in ethanolic leaf extract of Blepharis maderaspatensis (L) B. *World Jounrla of Pharmaceutical Research*. 2014; 3(9): 405-14. Available from: https://wjpr.s3.ap-south-1.amazonaws.com/article_issue/1414823052.pdf. [Accessed 11th October 2024].

[67] Abd El Baky HH, El-Baroty GS, Ibrahim EA. Antiproliferation and antioxidant properties of lipid extracts of the microalgae Scenedesmus obliquus grown under stress conditions. *Der Pharma Chemica*. 2014; 6(5): 24-34. Available from: https://www.researchgate.net/publication/286266869_Antiproliferation_and_antioxidant_properties_of_lipid_extracts_of_the_microalgae_Scenedesmus_obliquus_grown_under_stress_conditions. [Accessed 11th October 2024].

[68] Singab AN, Ibrahim N, Elsayed AE, El-Senousy W, Aly H, Abd Elsamiae A et al. Antiviral, cytotoxic, antioxidant and anti-cholinesterase activities of polysaccharides isolated from microalgae Spirulina platensis, Scenedesmus obliquus and Dunaliella salina. *HSPI*. Ain Shams University 2018; 2(2):121-37. doi:10.21608/aps.2018.18740.

[69] Custódio L, Soares F, Pereira H, Barreira L, Vizetto-Duarte C, Rodrigues MJ et al. Fatty acid composition and biological activities of Isochrysis galbana T-ISO, Tetraselmis sp. and Scenedesmus sp.: possible application in the pharmaceutical and functional food industries. *Journal of Applied Phycology*. 2014; 26(1):151-61. doi:10.1007/s10811-013-0098-0.

[70] Zaharieva MM, Zheleva-Dimitrova D, Rusinova-Videva S, Ilieva Y, Brachkova A, Balabanova V et al. Antimicrobial and Antioxidant Potential of Scenedesmus obliquus Microalgae in the Context of Integral Biorefinery Concept. *Molecules*. 2022; 27(2). doi:10.3390/molecules27020519.

[71] Reyna-Martinez R, Gomez-Flores R, López-Chuken U, Quintanilla-Licea R, Caballero-Hernandez D, Rodríguez-Padilla C et al. Antitumor activity of Chlorella sorokiniana and Scenedesmus sp. microalgae native of Nuevo León State, México. *PeerJ*. 2018; 6:e4358. doi:10.7717/peerj.4358.

[72] Ördög V, Stirk WA, Lenobel R, Bancířová M, Strnad M, van Staden J et al. Screening microalgae for some potentially useful agricultural and pharmaceutical secondary metabolites. *Journal of Applied Phycology*. 2004; 16(4): 309-14. doi:10.1023/B:JAPH.0000047789.34883.aa.

[73] Khadem S, Marles RJ. Monocyclic phenolic acids; hydroxy- and polyhydroxybenzoic acids: occurrence and recent bioactivity studies. *Molecules*. 2010; 15(11): 7985-8005. doi:10.3390/molecules15117985.

[74] Ávila-Román J, Talero E, Los Reyes C de, Zubía E, Motilva V, García-Mauriño S. Cytotoxic Activity of Microalgal-derived Oxylipins against Human Cancer Cell lines and their Impact on ATP Levels. *Natural*

Product Communications. 2016; 11(12): 1871-5. doi:10.1177/1934578X1601101225.

[75] Kagan ML, Matulka RA. Safety assessment of the microalgae Nannochloropsis oculata. *Toxicology Reports*. 2015; 2:617-23. doi:10.1016/j.toxrep.2015.03.008.

[76] Neumann U, Derwenskus F, Gille A, Louis S, Schmid-Staiger U, Briviba K et al. Bioavailability and Safety of Nutrients from the Microalgae Chlorella vulgaris, Nannochloropsis oceanica and Phaeodactylum tricornutum in C57BL/6 Mice. *Nutrients*. 2018; 10(8). doi:10.3390/nu10080965.

[77] Venkatraman A, Moovendhan M, Chandrasekaran K, Ramesh S, Albert A, Panchatcharam S et al. Alcoholic concentrate of microalgal biomass modulates cytotoxicity, apoptosis, and gene expression studied in hepatocellular carcinoma. *Biomass Conversion and Biorefinery*. 2022. doi:10.1007/s13399-022-02786-6.

[78] Sanjeewa KKA, Fernando IPS, Samarakoon KW, Lakmal HHC, Kim E-A, Kwon O-N et al. Anti-inflammatory and anti-cancer activities of sterol rich fraction of cultured marine microalga Nannochloropsis oculata. *ALGAE*. 2016; 31(3): 277-87. doi:10.4490/algae.2016.31.6.29.

[79] Kagan ML, Sullivan DW, Gad SC, Ballou CM. Safety assessment of EPA-rich polar lipid oil produced from the microalgae Nannochloropsis oculata. *International Journal of Toxicology*. 2014; 33(6): 459-74. doi:10.1177/1091581814553453.

[80] Custódio L, Soares F, Pereira H, Rodrigues MJ, Barreira L, Rauter AP et al. Botryococcus braunii and Nannochloropsis oculata extracts inhibit cholinesterases and protect human dopaminergic SH-SY5Y cells from H2O2-induced cytotoxicity. *Journal of Applied Phycology*. 2015; 27(2):839-48. doi:10.1007/s10811-014-0369-4.

[81] İnan B, Çakır Koç R, Özçimen D. Comparison of the anticancer effect of microalgal oils and microalgal oil-loaded electrosprayed nanoparticles against PC-3, SHSY-5Y and AGS cell lines. *Artificial Cells, Nanomedicine, and Biotechnology*. 2021; 49(1): 381-9. doi:10.1080/21691401.2021.1906263.

[82] Bhagavathy S, Sumathi P. Evaluation of antigenotoxic effects of carotenoids from green algae Chlorococcum humicola using human lymphocytes. *Asian Pacific Journal of Tropical Biomedicine*. 2012; 2(2):109-17. doi:10.1016/S2221-1691(11)60203-7.

[83] Office P. Commission Implementing Regulation (EU) 2017/2470 of 20 December 2017 establishing the Union list of novel foods in accordance with Regulation (EU) 2015/2283 of the European Parliament and of the Council on novel foods. 2017. (vol 60). Available from: http://data.europa.eu/eli/reg_impl/2017/2468/oj. [Accessed 11th October 2024].

[84] Mantecón L, Moyano R, Cameán AM, Jos A. Safety assessment of a lyophilized biomass of Tetraselmis chuii (TetraSOD®) in a 90 day feeding study. *Food and Chemical Toxicology*. 2019; 133:110810. doi:10.1016/j.fct.2019.110810.

[85] Rosa A, Deidda D, Serra A, Deiana M, Dessi MA, Pompei R. Omega-3 fatty acid composition and biological activity of three microalgae species. *Journal of Food, Agriculture & Environment*. 2005; 3(2): 120-4. Available from: https://www.academia.edu/63537470/Omega_3_fatty_acid_composition_and_biological_activity_of_three_microalgae_species. [Accessed 11th October 2024].

[86] Listenberger LL, Ory DS, Schaffer JE. Palmitate-induced apoptosis can occur through a ceramide-independent pathway. *Journal of Biological Chemistry*. 2001; 276(18): 14890-5. doi:10.1074/jbc.M010286200.

[87] Yao H-R, Liu J, Plumeri D, Cao Y-B, He T, Lin L et al. Lipotoxicity in HepG2 cells triggered by free fatty acids. *American Journal of Translational Research*. 2011; 3(3): 284-91. Available from: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3102573/. [Accessed 11th October 2024].