

***In Vitro* Cytotoxicity and Cell Migration Activities of Lipid Extracted from *Chlorella Sorokiniana* Shihira & R.W.Krauss Exposed to Nitrogen Stress**

Betül YILMAZ ÖZTÜRK^{*1}, Bükay YENİCE GÜRSU², Halima AL-THAWR³, İlknur DAĞ⁴

^{1,2,4}Central Research Laboratory Application and Research Center, Eskisehir Osmangazi University, Eskisehir, Türkiye

³Eskisehir Technical University, Faculty of Engineering, Department of Environmental Engineering, Eskisehir, Türkiye

⁴Vocational Health Services High School, Eskisehir Osmangazi University, Eskisehir, Türkiye

(Alınış / Received: 07.12.2024, Kabul / Accepted: 24.05.2025, Online Yayınlanma / Published Online: 25.08.2025)

Keywords:

Chlorella sorokiniana,
Nitrogen starvation,
Lipid accumulation,
Cell migration

Abstract: Microalgae contain valuable biochemical components and have the potential to be used in many areas. Depending on the culture conditions and species, the biochemical composition of microalgae also varies. Nitrogen is one of the most important nutrient sources for microalgae growth, and lipid accumulation in microalgae such as *Chlorella* increases significantly under stress conditions such as nitrogen limitation. Although many different studies have been conducted on the potential use of biochemical components of microalgae, information on the effects and mechanisms of microalgal lipids on cell viability, migration and wound healing is very limited. In this study, lipid extraction was carried out from *C. sorokiniana* microalga by dichloromethane/methanol method in the presence and absence of nitrogen. *In vitro* cytotoxic effects of both oils on the viability of L929 fibroblast cell line were investigated at eight different concentrations (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 µg/mL) using the WST-8 test. No toxic effect was found at any of the studied doses and the most effective and lowest dose that increased viability was determined to be 15.62 µg/ml. *In vitro* cell scratch test results revealed that lipid extract significantly accelerated cell migration and proliferation under nitrogen starvation compared to the control group. In both cases, no toxic effect of the obtained oil on the cells was observed. It is important to elucidate the therapeutic potential of these oils with detailed studies and to elucidate the changes in the oil composition of microalgae under stress conditions with further analyses.

Azot Stresine Maruz Kalan *Chlorella Sorokiniana* Shihira & R.W.Krauss 'dan Ekstrakte Edilen Yağın Yara İyileştirme Özelliklerinin Değerlendirilmesi

Anahtar kelimeler:

Chlorella sorokiniana,
Nitrojen açlığı,
Lipid birikimi,
Hücre göçü

Öz: Mikroalgler değerli biyokimyasal bileşenler içerir ve pekçok alanda kullanıma potansiyeline sahiptir. Kültür koşullarına ve türe bağlı olarak mikroalglerin biyokimyasal bileşimi de değişmektedir. Azot, mikroalg büyümesi için en önemli besin kaynaklarından biridir ve *Chlorella* gibi mikroalglerde lipid birikimi, azot sınırlaması gibi stres koşulları altında önemli ölçüde artar. Mikroalglerin biyokimyasal bileşenlerinin potansiyel kullanımı hakkında birçok farklı çalışma yapılmış olmasına rağmen, mikroalg lipidlerinin hücre canlılığı, göçü ve yara iyileşmesi üzerindeki etkileri ve mekanizmaları hakkında bilgiler çok sınırlıdır. Bu çalışmada, *C. sorokiniana* mikroalginden diklorometan/metanol yöntemi ile azot varlığında ve yokluğunda lipid ekstraksiyonu gerçekleştirilmiştir. Her iki yağın sekiz farklı konsantrasyonda (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 µg/mL) L929 fibroblast hücre hattının canlılığı üzerindeki *in vitro* sitotoksik etkileri WST-8 testi kullanılarak araştırılmıştır. Çalışılan hiçbir dozda toksik etki bulunmamış ve canlılığı artıran en etkili ve en düşük dozun 15.62 µg/ml olduğu belirlenmiştir. *In vitro* hücre çizik testi sonuçları, lipid özütünün azot açlığı altında kontrol grubuna

kıyasla hücre göçünü ve çoğalmasını önemli ölçüde hızlandırdığını ortaya koymuştur. Her iki durumda da elde edilen yağın hücreler üzerinde toksik etkisi gözlenmemiştir. Bu yağların terapötik potansiyelinin detaylı çalışmalarla açıklanması ve stres durumunda mikroalglerin yağ kompozisyonunda meydana gelen değişikliklerin ileri analizlerle aydınlatılması önemlidir.

1. Introduction

Microalgae, considered as the primary producers of the aquatic ecosystem, convert carbon dioxide in the atmosphere into valuable biomolecules such as proteins, lipids, carbohydrates or carotenoids [1]. They can adapt to various ecological environments, including wastewater, due to their rapid growth, high photosynthetic efficiency and simple growth requirements [2,3]. The oil content and production capacity of microalgae are quite high and changes in growth conditions can affect the quantity and quality of lipids. In particular, it is reported that nitrogen limitation increases the lipid content of microalgae [4]. Nitrogen is necessary for the growth of microalgae and plays a critical role in events such as DNA, protein and chlorophyll synthesis [3]. In fact, nutrient stress inhibits cell growth, but some microalgae species can synthesize fatty acids known as triglycerides (TGA), which do not participate in lipid formation in the cell membrane, by remodeling membrane lipids under conditions such as nitrogen limitation [3,5,6]. TAG serves both as an energy and carbon storage compound and as an electron sink under unfavorable conditions for growth [7].

Microalgae oil production can be enriched depending on the relevant species and environmental conditions. Various species belonging to the genera *Chlorella*, *Dunaliella* and *Scenedesmus* are reported as microalgae with high oil content (10-67%) [1]. In literature, it is reported that lipid droplets, cell volume, carotenoid amount and carbohydrate content increase during nitrogen limitation in green algae; general cell division stops due to decrease in protein, chlorophyll and total biomass levels [8]. In recent years, *C. sorokiniana* has attracted attention as a green microalga with high lipid content under different environmental growth environments. It is reported that this species accumulates more neutral lipids in nitrogen limitation compared to nitrogen-complete environmental conditions, but total biomass and energy products decrease due to increased damage to photosystem II [9].

Microalgal extracts have a high potential for use in various biomedical applications due to the bioactive components they contain, such as pigments, fatty acids or polysaccharides. These bioactive components show promising results in terms of antioxidant, anti-inflammatory, antibacterial, skin regenerative or wound healing applications [10]. Wound healing is a multi-stage process that involves the restoration of the structural and physical integrity of damaged

tissue. The healing process involves stages such as homeostasis, inflammation, migration, proliferation and remodeling [11,12]. In an in vitro study and animal model, microalgae were reported to increase the proliferation and migration of skin cells and accelerate the wound healing process [13]. In recent years, promising anti-inflammatory, antioxidant, antimicrobial and antiproliferative effects of lipids obtained from microalgae have begun to be revealed, but the relationships between the structure of bioactive lipids, their toxicity to healthy cells and their effects on cell migration have not yet been fully elucidated [13,14, 15]. In this study, the oil produced by *C. sorokiniana* microalga under nitrogen starvation and in a non-nitrogen deficient environment was extracted by dichloromethane/methanol method and the effects of these oils on the viability of fibroblast cell lines at different concentrations were evaluated using the WST-8 test. After determining the most effective low dose, it was aimed to compare the effects of the oils extracted at the relevant concentration in both cases on *in vitro* cell migration by cell scratch test.

2. Material and Method

2.1. Cultivation and growth condition of algal cells

The microalga strain used in the study, *C. sorokiniana*, was originally isolated from Musaözü Lake in Eskişehir and maintained from Shihira & R.W. Krauss, Osmangazi University (ESOGÜ) Biotechnology Laboratory. This strain was cultured in standard BG-11 medium, prepared according to our previous study [16]. The medium was formulated using stock solutions as follows:

Stock solutions (per 100 mL distilled water):

NaNO₃: 15 g, K₂HPO₄: 0.4 g, MgSO₄·7H₂O: 0.75 g, CaCl₂·2H₂O: 0.36 g, Citric acid: 0.06 g, Iron (III) ammonium citrate: 0.06 g, Na₂-EDTA: 0.01 g, Na₂CO₃: 0.2 g.

Final medium preparation (per 1 liter distilled water):

10 mL of each stock solution was added, including NaNO₃ (final concentration: 1.5 g/L), and 1 mL of the trace elements stock solution containing H₃BO₃: 61 mg, MnSO₄·H₂O: 169 mg, ZnSO₄·7H₂O: 287 mg, CuSO₄·5H₂O: 2.5 mg, (NH₄)₆Mo₇O₂₄·4H₂O: 12.5 mg. The pH of the final medium was adjusted to 6.8. [17,18]. The relative humidity conditions of the culture medium were set at 70-76%. Liquid cultures were inoculated at 2x10⁵ cells/mL and grown in BG-11 medium using the Multi-Cultivator OD-1000 (Photon Systems Instruments, Drásov, Czech

Republic) equipped with an AC-710 cooling unit and an LED array.

C. sorokiniana was cultured for 15 days under nitrogen stress in 2 growth tubes, each with a capacity of approximately 85 mL. Two experimental groups without nitrogen deprivation (Nitrogen 100%, as much as the content of BG11 medium) and with it (Nitrogen 0%, modified BG11 medium without nitrogen) were included in the study. The culture temperature was set at 28 °C, and white light was used with wavelengths between 430-750 nm. The light intensity is 200 $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$. The carbon dioxide required for photosynthesis came from compressed atmospheric air reaching the photobioreactor, which was continuously ventilated at approximately equal flow rates at a rate of 1 volume of gas per liquid volume per minute (vvm). To maintain sterilization, the air flow was passed through 0.2 μm PTFE membrane filters (Fluoropore, Merck Millipore).

2.2. Lipid extraction

Lipid extraction was carried out with biomass produced within 15 days after algal cell cultivation and by the dichloromethane/methanol method [19]. Initially, nearly 60 mg of wet microalgae biomass sample was kept in an ultrasonic water bath for 60 minutes in order to disrupt the cell walls. Samples were treated with a 2:1 dichloromethane/methanol mixture. The suspension was then centrifuged at 10,000 rpm, 20 min, and 4 °C to obtain two phases. The first phase (located in the upper part) contains lipids dissolved in the solvent, while the second phase (located in the lower part) belongs to proteins and carbohydrates. After the subphase was removed, the lipid samples were placed in an oven at 60 °C for 24 h to ensure solvent elimination. Based on the ratio of the total amount of lipid extracted to the wet weight of the algae, the lipid extraction efficiency (1) was calculated according to the following formula [20]:

$$\text{Lipid yield (\%)} = \text{g of oil/dry weight of biomass (1)}$$

2.3. Cytotoxicity test

Preparation of Cell Line Culture Medium:

One fibroblast L929 cell line with passage number 16 was used for the study (L929 Cell line originates from ATCC CCL-1). Cells were grown in Eagle's Minimum Essential Medium (EMEM, Multicell, 320-005) containing 10% Fetal bovine serum (FBS, Pan Biotech P30-1301), in a 25T Flask, by checking twice a day; It was expected to reach confluency of 70% or above. (Doubling Time: 22-26 hours).

Preparation of Lipid Samples

Samples were prepared at a concentration of 10 mg/mL. After exposure to UV light for 30 minutes, 100 μL Dimetil Sülfoksit (DMSO, Sigma-Aldrich, D2650)

was dissolved in the medium and homogenization was achieved with the medium. The concentrations to be used after this process were diluted with complete medium.

Planting the Cells in 96-Well Plates

In a 25T flask, 500 μL Trypsin-EDTA (Gibco, 15400054) was added to the cells that reached 70% or more confluency and kept for 3-5 minutes at 37°C in a 5% CO₂ environment. As soon as the cells were observed to be dissociated under an inverted microscope (Zeiss Primovert, Germany), medium containing 10% FBS was added. After centrifugation at 300xg for 5 minutes, counting was done on the Logos Luna II (Logos Biosystems, Anyang, South Korea) device using Trypan blue (Gibco, 1525061), and 10% FBS medium was added to obtain 10⁴ cells per well.

Application of Cytotoxicity Test

When 70% confluency was reached in each well, the medium in the culture dish was withdrawn. Then, after adding fresh medium, the samples were added to the cell dish. Each group was made in at least 3 repeat wells to ensure repeated analysis. Eight different concentrations of active ingredient were applied to the cells (500, 250, 125, 62.5, 31.25, 15.62, 7.81, 3.90 $\mu\text{g/mL}$). After exposure, the cells are waited for 22 h without being removed from the incubator. Apart from the test samples, the so-called negative well contained only medium and cells (Growth control). Dichloromethane (DCM), which is frequently used for lipid extraction, is generally not preferred in cell culture applications due to its cytotoxic and mutagenic effects [21]. For this reason, DMSO was used as the solvent in our study. DMSO is widely used in cell culture studies when applied at final concentrations below 1% and does not show toxic effects. It has also been shown to provide effective solubilization of lipid extract while preserving cell viability and is compatible with various cell types, including L929 [22,23]. Solvent used for lipid dissolution: After lipid extraction, the remaining oil was dissolved in 100 μL of DMSO to ensure proper solubilization before application to cell culture. This volume of DMSO was chosen based on preliminary tests showing that it was sufficient to completely dissolve 10 mg of extracted oil and achieve a homogeneous distribution in the culture medium. The solubility and homogeneity of the oil in DMSO were confirmed by visual inspection and pipetting consistency. It was ensured that the final DMSO concentration in all experimental groups remained below 1%, and a separate solvent control group was not included in the study since the effect of this rate on cell viability was negligible. The positive control well contained 2 μL hydrogen peroxide (H₂O₂, Merck, H1009) (a drug control with known toxicity) in addition to the medium and cells. Finally, blank wells were prepared by adding only the medium. At the end of 22 hours, 10% of the well volume (equal to 20 μL Enhanced Cell Counting Kit 8, WST-8 (E-CK-A362) in the experiment) of WST-8 solution was added. The

lights were turned off during this process. The cell culture dish was wrapped with aluminum foil and kept in the incubator for another 2 h. At the end of 2 h, spectrophotometric readings were made in the cell culture dish at wavelengths of 450 nm and 630 nm [24]. The results were formulated and percent cell survival was determined as presented below (Eq. 2):

$$\% \text{Viability} = \frac{(\text{Experimental well} - \text{Blank})}{(\text{Negative control} - \text{Blank})} * 100 \quad (2)$$

2.4. *In vitro* cell scratch assay

To determine the effects of the oil extracts on *in vitro* cell migration, cell scratch test was performed using L929 fibroblast cells. The samples were planted in 48-well tissue culture dishes in EMEM medium with 10% FBS, 1% penicillin-streptomycin (Gibco, 15070063) and 4 mM L-glutamine (Sigma- Aldrich, G8540) at a concentration of 2×10^4 cells/ml and waited until the surface was covered in a monolayer. Then, an *in vitro* scratch model was created by scratching the surface in one move from one end to the other with the help of a sterile pipette tip (200 μ l). During the process, the upper medium was removed and washed with PBS to remove the shielded cells. After the cytotoxicity experiment, the biomaterial produced at the determined doses was applied and incubated for 24 h at 37 °C in a 5% CO₂ incubator [25]. The solvent of the solvent extract without biomaterial was added to the negative control wells. *In vitro* scratch model was viewed under an inverted microscope (Zeiss Primovert) and at least two images were taken from each well and recorded as hour 0. Likewise, the samples were digitally photographed after 24 h in order to make the necessary calculations. The captured images were analyzed using the Image J image analysis program. For this purpose, the entire area of the image and relative migration areas were calculated in the program. The migration area percentage (Eq. 3) was calculated using the following formula:

$$\text{Area percentage for migration determination} = \left[\frac{(\text{Area}_{t0} - \text{Area}_{t24})}{\text{Area}_{t0}} \right] \times 100 \quad (3)$$

Here, Area_{t0} refers to the measured area of the photo taken at the beginning, and Area_{t24} refers to the area of the photo taken at the 24th h.

2.5. Statistical analyses

The data were presented as mean values with standard deviations (SD), and each experiment was carried out separately in triplicate. Prior to executing a one-way ANOVA, the data's normal distribution was confirmed using the Shapiro-Wilk test, and the equality of variances between groups was confirmed using Levene's test. To find statistically significant differences between the experimental and control groups, Tukey's HSD post hoc analysis was used once one-way ANOVA was completed and these

presumptions were met. A significance level of $p < 0.05$ was used throughout the analysis, and SPSS software (version 26) was used for all statistical evaluations.

3. Results

In our study, the total amount of oil extracted from *C. sorokiniana* microalga in an environment without nitrogen stress (OE-NS) was calculated as 0.019 g and the lipid yield was calculated as 31.7%, while the total amount of oil obtained in the case of nitrogen stress (OE-S) was calculated as 0.078 g and the lipid yield was calculated as 130% (Table 1). Here, lipid extraction efficiency was calculated as the ratio of the extracted lipid weight to the dry biomass weight by following a standard approach (Eq. 1). The 130% lipid yield reported under nitrogen deprivation (0% nitrogen) conditions was relative to the control group (100% nitrogen condition) where the lipid yield was calculated as 31.7%. Therefore, 130% efficiency indicated a 4.1-fold increase in the extracted oil compared to the full nitrogen (100%) condition, indicating significant lipid accumulation under nitrogen stress. In addition, this difference in lipid yield between the groups was statistically significant ($p < 0.05$).

Table 1. Amount and productivity of lipid obtained from *C. sorokiniana*

Algal biomass (mg)	Extracted Lipid (g)	Lipid Yield (%)
Nitrogen 100% 60	0.019	31.7
Nitrogen 0% 60	0.078	130

* Nitrogen 100%, OE-NS: Prepared with normal BG-11 medium under non-stressed conditions.

**Nitrogen 0%, OE-S: The BG-11 medium was modified and defined as a nitrogen-free condition.

In line with the cytotoxicity analysis data, it was determined that both oils used in the study were not toxic to the cells. Figure 1 shows the percentage viability of oil obtained from OE-NS microalga and applied at eight different concentrations on L929 cells. It was determined that the groups with higher percent viability compared to the control group belonged to concentrations of 62.5, 31.25 and 15.62 μ g/mL, and 15.62 μ g/mL, the lowest effective dose among these three concentrations, was selected to be included in the subsequent *in vitro* cell scratch study. These differences among OE-NS treatment groups were statistically significant according to Tukey's HSD test ($p < 0.05$).

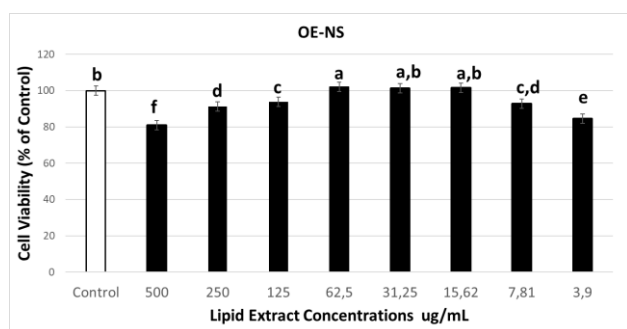


Figure 1. Percentage survival rates of lipid obtained from *C. sorokiniana* microalga without nitrogen stress (OE-NS) and applied at 8 different concentrations on L929 fibroblast cells. Different lower case letters indicate that the concentrations for each OE-NS were significantly different from each other according to Tukey's HSD test ($p < 0.05$).

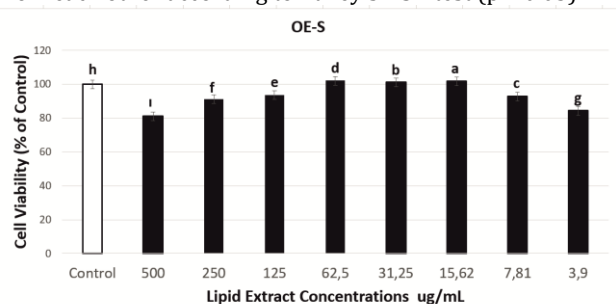


Figure 2. The percentage viability rates of lipid obtained from nitrogen stressed (OE-S) microalga and applied at 8 different concentrations on L929 cells. Different lower case letters indicate that the concentrations for each OE-S were significantly different from each other according to Tukey's HSD test ($p < 0.05$).

In this group, similar to the OE-NS group, the groups with higher percent viability compared to the control group were at concentrations of 62.5, 31.25 and 15.62 µg/mL, and 15.62 µg/mL, the lowest effective dose among these three concentrations, was selected to be included in the subsequent *in vitro* cell scratch study. These differences among OE-S treatment groups were also statistically significant ($p < 0.05$, Tukey's HSD test).

Cell images obtained with an inverted microscope (Zeiss Primovert, Germany) as a result of 0 and 24 h applications of the oil at a dose of 15.62 µg/mL, which was selected to be used for both OE-NS and OE-S groups in the cell scratch test, are shown in Figure 3 and the percentage cell migration rates are presented in Figure 4. According to the data obtained, there was a 45% wound closure at the 24th hour compared to the 0th hour in the control group where no active ingredient was applied. As a result of application to adjacent cells obtained from OE-NS group, a greater cell migration was observed at the 24th hour compared to the control and this rate was measured as 55%. As a result of applying the extracted oil to the cells in case of OE-S group, a 96% cell migration was calculated at the 24th hour compared to the control (Figure 3). There was a statistically significant difference in cell migration between all groups ($p < 0.05$).

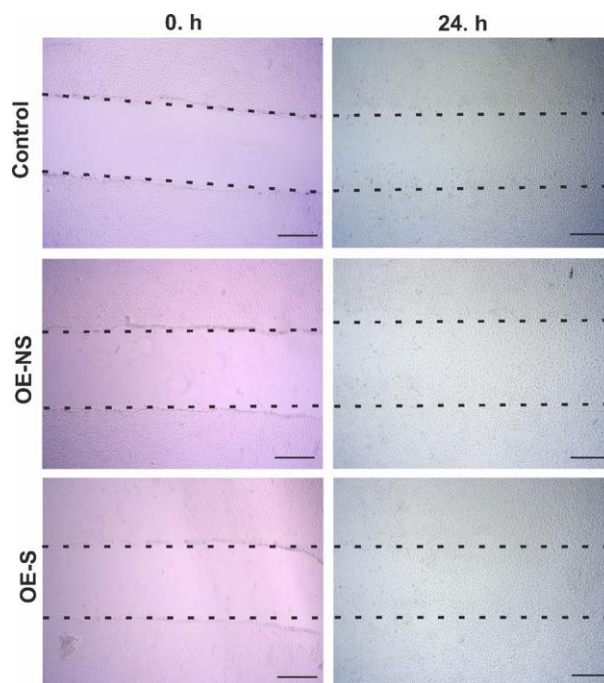


Figure 3. Inverted microscope images obtained as a result of the application of lipid obtained from *C. sorokiniana* microalga at a dose of 15.62 µg/mL on L929 cell lines at 0 and 24 h under conditions OE-NS (without nitrogen stress) and OE-S (nitrogen stressed) compared to the control group (Scale bar: 500 µm).

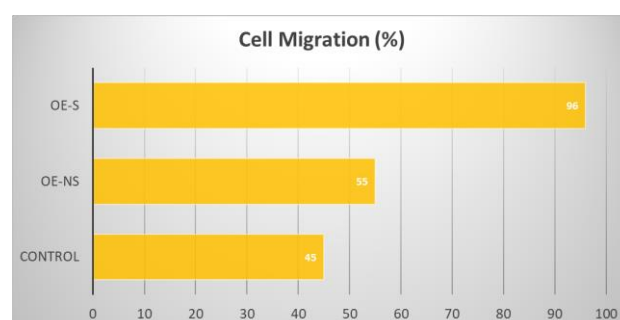


Figure 4. Percentage cell migration rates obtained from the 24 h cell scratch test of the lipid obtained from *C. sorokiniana* microalga and applied to L929 cell lines at a dose of 15.62 µg/mL for OE-NS (without nitrogen stress) and OE-S (nitrogen stressed) groups.

The high cell migration effect of the oil extracted under nitrogen stress in the scratch test was clearly observed at higher magnification and this is presented in Figure 5.

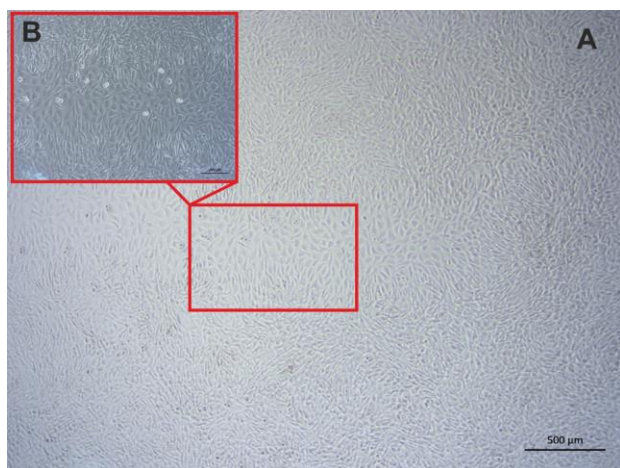


Figure 5. *In vitro* cell migration effect observed after the application of the lipid extract obtained from *C. sorokiniana* microalga exposed to nitrogen starvation with the cell stretching test.

4. Discussion and Conclusions

The fact that microalgae do not require a large area for development and can easily adapt to adverse stress conditions makes them an ideal alternative for the production of various bioactive molecules. *Chlorella* microalgae have also attracted great attention in recent years due to their capacity to synthesize large amounts of valuable bioactive compounds. Compared to other microalgae, *Chlorella* species can synthesize more bioactive compounds and are therefore one of the most studied and cultured microalgae worldwide [26].

Oils are one of the most important energy sources of microalgae, and they both participate in the cell membrane structure and mediate various cell signaling systems when environmental conditions change. Microalgae used for lipid production are divided into two categories; some microalgae have high lipid content but low cell growth, while the other group has high cell growth but low lipid content. In previous studies, microalgae with high lipid content have been studied frequently, but since this group grows slowly, oil production is also low. On the other hand, *Chlorella vulgaris* microalgae show rapid growth (short doubling time 19 h), while lipid accumulation content is only 20% [27]. In the literature, it has been reported that some microalgae such as *Chlorella* increase in lipid content and production under various abiotic stress conditions [28].

Nitrogen is one of the most essential nutrients affecting the growth and lipid accumulation of microalgae. Nitrogen starvation constitutes a critical stress source for microalgae and causes significant changes in the cellular metabolism and development of the organism [26]. When there is no nitrogen required for protein synthesis in the environment, excess carbon from photosynthesis is directed to storage molecules such as triglycerides or starch.

Nitrogen stress in *Chlorella* in particular can increase the lipid and triglyceride content by two to three times [29,30]. More energy can be produced from carbohydrates through the accumulation and oxidation of TAG in the cell. Thus, the cell has an effective reserve when it recovers from deprivation [31]. The increase in the total lipid content of algal cells under stress is generally also associated with an increase in reactive oxygen species (ROS), but the regulatory mechanism of ROS on lipid accumulation has not been elucidated [32]. However, lipid production in microalgae varies depending on factors such as microalgae species, cultivation conditions, nutrient availability, light, pH or temperature [33, 34].

Since wound healing is a complex biological process, there are several *in vitro* and *in vivo* tests that offer a first perspective to evaluate various natural products for their *in vitro* wound healing potential [35,36,37]. Among these, the scratch test stands out as a valuable, cheap and simple method. This test is associated with the second phase of wound healing, expressed by the proliferation and migration of keratinocytes and fibroblasts [34,38].

Microalgal lipids consist of polar (phospholipids etc.) and neutral lipids (acylglycerol, free fatty acids etc.). In the exponential growth phase, microalgae are mostly rich in polar lipids, while in the stationary phase, TAG accumulation is observed due to stress conditions where nutrients are limited [34]. This study includes the *in vitro* comparison of the effects of lipids obtained from *C. sorokiniana* microalga in the presence and absence of nitrogen on the viability and cell migration of fibroblast cell lines. In our study, a 15-day period was chosen for nitrogen stress based on previous studies showing that prolonged nitrogen deprivation can lead to significant lipid accumulation in *C. sorokiniana*. For instance, a study by Zhang et al. (2013) observed substantial neutral lipid accumulation in *C. sorokiniana* C3 cells after 8 days of nitrogen starvation [9]. Similarly, research by Negi et al. (2016) reported that *C. sorokiniana* maintained growth rates and accumulated neutral lipids over a two-week nitrogen deprivation period [39]. These findings suggest that prolonged nitrogen stress can effectively induce lipid accumulation without severely compromising cell viability.

Our observations align with the aforementioned studies, indicating that *C. sorokiniana* cells remained viable after the 15-day nitrogen stress treatment. Despite the nutrient deprivation, the cells continued to exhibit metabolic activity, which is consistent with previous reports demonstrating that *C. sorokiniana* can sustain growth and viability under extended nitrogen-limited conditions.

Our data supported that the obtained lipids did not show toxicity on the fibroblast cell line and had a

positive effect on cell migration. The obtained lipid increased the migration of cells and also the population of L929 cells in the scratch area due to the proliferation of migrating cells. This increase occurred at a higher rate in the case of nitrogen starvation.

There is very limited data in the literature explaining the effects of bioactive components obtained from microalgae, especially oils, on cell migration and wound healing. In a recent *in vivo* study, it was reported that fatty acids extracted from the microalga *Parachlorella kessleri* had a significant effect in reducing excisional wounds and burns [40]. In another study, it was shown that lipid extract obtained from the microalga *Nannochloropsis oceanica* had the capacity to regenerate keratinocytes previously exposed to UVB radiation [41]. Nascimento-Neto and colleagues reported the wound healing, high collagen densification and epithelial restructuring effects of lectin obtained from the macroalga *Bryothamnion seaforthii* [42]. In a study by De Melo et al., *in vivo* wound healing potential of a hydrogel-based *C. vulgaris* extract was investigated. For this purpose, a hydrogel formulation with different concentrations of *C. vulgaris* extracts grown under autotrophic and mixotrophic conditions was prepared and its effects on excisional wounds in mice were evaluated. When the effects of the extracts were considered in the study in terms of protein concentration, phytochemical profile, hemagglutination activity, antioxidant activity and antibacterial activity, it was reported that the extract obtained in mixotrophic conditions accelerated healing and showed anti-inflammatory properties [38]. In a similar study, it was stated that necrotic tissue could be removed, inflammatory process could be modulated and re-epithelialization process could be initiated with the help of collagenolytic enzyme in the extract obtained from *C. vulgaris* UTEX 1803 [43]. Our findings do not examine a complete wound healing process as they only examined fibroblast migration, but they reveal the importance of investigating the *in vitro* cell migration potential of lipids obtained from *C. sorokiniana* against L929 cells stimulated with scratch test and as a possible wound healing component.

As a result, this study involves the initial investigation of the effects of oil produced by *C. sorokiniana* microalga on *in vitro* cell migration compared to the control group under nitrogen stress. Our results showed that the oil extracted from *C. sorokiniana* microalga, which accumulates significant lipids under nitrogen stress, is non-toxic and its *in vitro* migration properties are significantly increased compared to the control group without nitrogen deficiency. However, detailed *in vitro* and *in vivo* data, possible mechanisms of action, and changes in oil composition are needed to elucidate the findings and reveal the wound healing properties.

Acknowledgments

This work was performed at Eskişehir Osmangazi University Central Research Laboratory Application and Research Center (ARUM).

Declaration of Ethical Code

In this study, we undertake that all the rules required to be followed within the scope of the "Higher Education Institutions Scientific Research and Publication Ethics Directive" are complied with, and that none of the actions stated under the heading "Actions Against Scientific Research and Publication Ethics" are not carried out.

References

- [1] Udayan, A., Pandey, A. K., Sharma, P., Sreekumar, N., & Kumar, S. 2021. Systems Microbiology and Biomanufacturing, 1(4), 411-431.
- [2] Goh, B. H. H., Ong, H. C., Cheah, M. Y., Chen, W. H., Yu, K. L., & Mahlia, T. M. I. 2019. Renewable and Sustainable Energy Reviews, 107, 59-74.
- [3] Şirin, P. A., Serdar, S. 2024. Folia Microbiologica, 1-14.
- [4] Huang, X., Huang, Z., Wen, W., & Yan, J. 2013. Journal of Applied Phycology, 25, 129-137.
- [5] Han, D., Jia, J., Li, J., Sommerfeld, M., Xu, J., & Hu, Q. 2017. Frontiers in Marine Science, 4, 242.
- [6] Fattore, N., Bellan, A., Pedroletti, L., Vitulo, N., & Morosinotto, T. 2021. Algal Research, 58, 102368.
- [7] Breuer, G., de Jaeger, L., Artus, V. G., Martens, D. E., Springer, J., Draaisma, R. B., Wijffels, R.H.W & Lamers, P. P. 2014. Biotechnology for Biofuels, 7, 1-11.
- [8] Cakmak T, Angun P, Demiray YE, Ozkan AD, Elibol Z, Tekinay T. 2012. Biotechnology and Bioengineering 109:1947–1957
- [9] Zhang, Y. M., Chen, H., He, C. L., Wang, Q. 2013. PloS one, 8(7), e69225.
- [10] Miguel, S. P., Ribeiro, M. P., Otero, A., & Coutinho, P. 2021. Algal Research, 58, 102395.
- [11] Guo,, S., DiPietro, L. A. 2010. Journal of Dental Research, 89(3), 219–229.
- [12] Rodrigues, M., Kosaric, N., Bonham, C. A., & Gurtner, G. C. 2019. Physiological Reviews, 99(1), 665–706.
- [13] Huang, H., Lang, Y., & Zhou, M. 2024. Algal Research, 103504.
- [14] Conde, T., Lopes, D., Łuczaj, W., Neves, B., Pinto, B., Maurício, T., Domingues, P.,

- Skrzydłowska, E., Domingues, M. R. 2022. *Metabolites*, 12(2), 96.
- [15] Conde, T., Lopes, D., Łuczaj, W., Neves, B., Pinto, B., Maurício, T., Skrzydłowska, E & Domingues, M. R. 2022. *Metabolites*, 12(2), 96.
- [16] Yılmaz Öztürk, B., Yenice Gürsu, B., Koyuncu, O., Dağ. 2023. Ultrastructural Investigation of the Effects of Malathion on the Green Alga *Chlorella sorokiniana* Shihira & R.W. Krauss," 6th National Congress of Biological Sciences, November 3-5, Eskişehir.
- [17] Fidan, E. C., Öztürk, B. Y., Şirin, Ü. 2018. *Biyolojik Çeşitlilik ve Koruma*, 11(1), 139-142.
- [18] Rippka, R. 1988. *Methods in enzymology*, vol 167. Academic Press, New York, pp 3-27.
- [19] Ren, X., Wei, C., Yan, Q., Shan, X., Wu, M., Zhao, X., Song, Y. 2021. *Scientific Reports*, 11(1), 20221.
- [20] Parida, S., Biswal, S. 2020. *International Journal of Energy Applications and Technologies*, 7(3), 69-73.
- [21] Buranarom, A., Navasumrit, P., Ngaotepprutaram, T., & Ruchirawat, M. 2021. *Chemico-Biological Interactions*, 346, 109580.
- [22] Galvao, J., Davis, B., Tilley, M., Normando, E., Duchon, M. R., & Cordeiro, M. F. 2014. *The FASEB Journal*, 8(3), 1317-1330.
- [23] Javaid, N., Patra, M. C., Seo, H., Yasmeen, F., & Choi, S. 2020. *International Journal of Molecular Sciences*, 21(24), 9450.
- [24] Park, C. M., & Xian, M. 2015. In *Methods in enzymology*, 554, 127-142.
- [25] Chen, S., bin Abdul Rahim, A. A., Mok, P., Liu, D. 2023. *BMC biotechnology*, 23(1), 32.
- [26] Fang, Y., Cai, Y., Zhang, Q., Ruan, R., & Zhou, T. 2024. *Process Safety and Environmental Protection*.
- [27] Griffiths, M. J., & Harrison, S. T. 2009. *Journal of applied phycology*, 21, 493-507.
- [28] Lv, J. M., Cheng, L. H., Xu, X. H., Zhang, L., & Chen, H. L. 2010. *Bioresource technology*, 101(17), 6797-6804.
- [29] Chen, H., & Wang, Q. 2021. *Biological Reviews*, 96(5), 2373-2391.
- [30] Converti, A., Casazza, A. A., Ortiz, E. Y., Perego, P., Del Borghi, M. 2009. *Chemical Engineering and Processing: Process Intensification*, 48(6), 1146-1151.
- [31] Xin, L., Hong-Ying, H., & Jia, Y. 2010. *New Biotechnology*, 27(1), 59-63.
- [32] Zhang, L., Liao, C., Yang, Y., Wang, Y. Z., Ding, K., Huo, D., Hou, C. 2019. *Bioresource technology*, 287, 121414.
- [33] Vello, V., Chu, W. L., Lim, P. E., Majid, N. A., Phang, S. M. 2018. *Journal of applied phycology*, 30, 3131-3151.
- [34] Morales, M., Aflalo, C., & Bernard, O. 2021. *Biomass and Bioenergy*, 150, 106108.
- [35] Boateng, J., Matthews, K. H., Stevens, H. N. E., & Eccleston, G. M. 2008. *Journal of Pharmaceutical Sciences*, 97(8), 2892-2923.
- [36] Masson-Meyers, D. S., Andrade, T. A., Caetano, G. F., Guimaraes, F. R., Leite, M. N., Leite, S. N., & Frade, M. A. C. 2020. *International journal of experimental pathology*, 101(1-2), 21-37.
- [37] Ibrahim, N. I., Wong, S. K., Mohamed, I. N., Mohamed, N., Chin, K. Y., Ima-Nirwana, S., & Shuid, A. N. 2018. *International journal of environmental research and public health*, 15(11), 2360.
- [38] Kaur, M., Bhatia, S., Gupta, U., Decker, E., Tak, Y., Bali, M., Gupta, V. K., Dar, R. A & Bala, S. 2023. *Phytochemistry Reviews*, 22(4), 903-933.
- [39] Negi, S., Barry, A. N., Friedland, N., Sudasinghe, N., Subramanian, S., Pieris, S., Holguin, F. O., Dungan, B., Schaub, T & Sayre, R. 2016. *Journal of Applied Phycology*, 28, 803-812.
- [40] El-Sheekh, M., Bedaiwy, M., Mansour, H., & El-Shenody, R. A. 2024. *Burns*, 50(4), 924-935.
- [41] Biernacki, M., Conde, T., Stasiewicz, A., Surazyński, A., Domingues, M. R., Domingues, P., & Skrzydłowska, E. 2023. *International Journal of Molecular Sciences*, 24(18), 14323.
- [42] Nascimento-Neto, L.G., Carneiro, R. F., Silva, S. R., Silva, B. R., Arruda, F. V. S., Carneiro, V. A., Nascimento, K. S., Saker-Sampaio, S., Silva, V. A Jr., Porto, A. L. F., Cavada, B. S., Sampaio, A. H., Teixeira, E. H., Nagano, C. S. 2012. *Marine Drugs*, 10, 1936-1954.
- [43] de Melo, R. G., de Andrade, A. F., Bezerra, R. P., Viana Marques, D. de A., da Silva, V. A., Paz, S. T., de Lima Filho, J. L., & Porto, A. L. F. 2019. *Journal of Applied Phycology*, 31(6).