REPUBLIC OF TÜRKİYE

YILDIZ TECHNICAL UNIVERSITY

GRADUATE SCHOOL OF SCIENCE AND ENGINEERING

CHARACTERIZATION AND ANTICANCER ACTIVITY OF THE *AUXENOCHLORELLA PYRENOIDOSA* OZCIMEN.001 AND *CHLORELLA SOROKINIANA* EGEMEN.002 POLAR MICROALGAE

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MASTER OF SCIENCE THESIS

Department of Bioengineering

Bioengineering Program

Supervisor

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July, 2023

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Gülcan Ayşin KARACA

Signature

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Dedicated to my family

and the ones who are

always by my side

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LIST OF SYMBOLS

α	Alpha
$AlCl_3$	Aluminum chloride
β	Beta
cm	Centimeter
cm^{-1}	Centimetre Power (–1)
$CuSO_4.5H_2O$	Copper sulfate pentahydrate
°	Degree
℃	Degree Celsius
d.nm	Diameter.nm
t_d	Doubling Time
DE	Dry Extract
DW	Dry Weight
GAE	Gallic Acid Standart
Au	Gold
g	Grams
hr	Hours
L	Liter
µg	Microgram
µL	Microliter
mg	Milligram
mm	Millimeter
mL	Milliliter
min	Minute
nm	Nanometer
NP	Nanoparticle
OD	Optical Density
Pd	Palladium
%	Percent
K	Potassium
Rpm	Revolutions per Minute

Na	Sodium
Na_2CO_3	Sodium Carbonate
NaOH	Sodium Hydroxide
μ	Specific Growth Rate
H_2SO_4	Sulfuric Acid
θ	Theta
Q	Quercetin Standart

LIST OF ABBREVIATIONS

A549	Lung Cancer Cell Line
AL	Alginate
ASH	Amount of Ash
ASTM	The American Society for Testing and Materials
ATR	Attenuated Total Reflection
BBM	Bold's Basal Medium
BSA	Bovine Serum Albumin
CP	*Auxenochlorella pyrenoidosa* Ozcimen.001
CP Ext	*A. pyrenoidosa* Ozcimen.001 extract
CP NP	*A. pyrenoidosa* Ozcimen.001 extract loaded nanoparticle
CS	*Chlorella sorokiniana* Egemen.002
CS Ext	*C. sorokiniana* Egemen.002 extract
CS NP	*C. sorokiniana* Egemen.002 extract loaded nanoparticle
DLS	Dynamic Light Scattering
DMEM	Dulbecco′s Modified Eagle′s Medium
DMSO	Dimethyl Sulfoxide
DPPH	1, 1-difenil-2-pikril hidrazil
EE	Encapsulation Efficiency
FAME	Fatty Acid Methyl Ester
FBS	Fetal Bovine Serum
FC	Amount of Fixed Carbon Content
FESEM	Field Emission Scanning Electron Microscopy
FID	Flame Ionization Detector
FTIR	Fourier Transform Infrared Spectroscopy
MCF-7	Breast Cancer Cell Line
PBS	Phosphate Buffered Saline
PDI	Polydispersity Index
PolReC	ITU Polar Research Application and Research Center
PVA	Polyvinyl Alcohol

TAEIII	Antarctic Science Expedition
TASE-I	Turkish Arctic Science Expedition
TFC	Total Flavonoid Content
TG	Thermogravimetry
TGA	Thermogravimetric Analysis
TPC	Total Phenolic Content
UV	Ultraviolet Light
Vis	Visible Light
VM	Amount of Volatile Matter
XRD	X-Ray Diffraction Analysis
XTT	2,3-Bis(2-metoksi4-nitro-5-sulfofenil)-2H-tetrazolyum-5 carboxanilide

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ABSTRACT

Characterization and Anticancer Activity of the *Auxenochlorella pyrenoidosa* Ozcimen.001 and *Chlorella sorokiniana* Egemen.002 Polar Microalgae

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Bioengineering Program

Master of Science Thesis

Supervisor: Prof. Dr. Didem ÖZÇİMEN

Microalgae obtained from the polar regions are one of the species that produce valuable bioactives and especially effective antioxidants as a metabolic adaptation mechanism to tolerate oxidative environmental circumstances. Flavonoids, which are natural pigments found in microalgae and synthesized as secondary metabolites, appear as a component that can be used against cancer cells. In the literature, studies on the anticancer activity of polar microalgae are limited and there are no studies related to the species used in this thesis.

In this context, first, cultivation of the polar microalgae species *Auxenochlorella pyrenoidosa* Ozcimen.001 (CP) and *Chlorella sorokiniana* Egemen.002 (CS) isolated from Antarctic and Arctic regions in laboratory were performed and then these cultures were characterized. After that, microalgae extracts obtained by using a Soxhlet apparatus are encapsulated via electrospraying. Then, XTT method was implemented on A-549 and MCF-7 cell lines to microalgae extract and nanoparticles for cell viability analysis.

As a result of the analyses conducted for the study, the flavonoid content of the CP and CS microalgae extracts was found to be 1.16 mg Q/g DE and 0.6 mg Q/g DE, respectively. The obtained CP nanoparticle's size and PdI value were determined to be 294.7 nm and 0.406, whereas the CS nanoparticle's size and PdI value were 156.4 nm and 0.40. Encapsulation efficiency of the produced nanoparticles was determined as 85-92%. As a result of anticancer analysis performed on the A-549 and MCF-7 cell lines in accordance with the study, it was shown that nanoparticles loaded with microalgae extract exhibited anti-cancer activity. As a result, it has been determined that the polar microalgae extract-loaded nanoparticles utilized in the direction of the data obtained from the study have the potential to be evaluated as an anticarcinogenic agent in the biotechnological drug category.

Keywords: Polar microalgae, nanoparticle, anti-cancer activity, electrospraying, encapsulation

ÖZET

Auxenochlorella pyrenoidosa Ozcimen.001 ve *Chlorella sorokiniana* Egemen.002 Kutup Mikroalglerinin Karakterizasyonu ve Antikanser Aktivitesi

Gülcan Ayşin KARACA

Biyomühendislik Anabilim Dalı

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Danışman: Prof. Dr. Didem ÖZÇİMEN

Mikroalg türleri içerisinde, kutup bölgelerinden izole edilen mikroalgler oksidatif çevre koşullarını tolere etmek amacıyla metabolik adaptasyon mekanizması olarak değerli biyoaktiflerin ve özellikle etkili antioksidanların biyosentezini gerçekleştirmektedir. Mikroalglerin zengin içeriklerinde bulunan doğal pigmentlerden olan ve ikincil metabolitler olarak sentezlenen flavonoidler kanser hücrelerine karşı kullanılabilecek bir bileşen olarak karşımıza çıkmaktadır. Literatürde kutup mikroalglerinin antikanser aktivitesi ile ilgili çalışmalar sınırlı sayıda olup bu tez çalışmasında kullanılan türler ile alakalı çalışma bulunmamaktadır.

Bu doğrultuda Antarktik ve Arktik bölgelerden izole edilen *Auxenochlorella pyrenoidosa* Ozcimen.001 (CP) ve *Chlorella sorokiniana* Egemen.002 (CS) kutup mikroalg türlerinin laboratuvar ortamında yetiştirilmesinden sonra kimyasal içerik analizleri gerçekleştirilmiştir. Daha sonrasında Soxhlet cihazı ile ekstraksiyona tabii tutulan mikroalg kültürlerinden elde edilen mikroalg ekstraktları elektrospreyleme yöntemi ile enkapsüle edilmiş ve elde edilen nanopartiküllerin karakterizasyon çalışmaları gerçekleştirilmiştir. Sonrasında hücre canlılık analizi

ekstraktlar ve nanopartiküller XTT yöntemi ile A-549 ve MCF-7 hücre hatlarına uygulanmıştır.

Çalışmada gerçekleştirilen analizler sonucu elde edilen CP ve CS mikroalg ekstraktlarının flavonoid miktarı sırasıyla 1,16 mg Q/g DE ve 0,6 mg Q/g DE olarak tespit edilmiştir. Elde edilen CP nanopartikülünün boyutu ve PDI değeri 294,7 nm ve 0,406 olarak ölçülürken, CS nanopartikülünün boyutu ve PDI değeri 156,4 nm ve 0,40 olarak ölçülmüştür. Üretilen nanopartiküllerin enkapsülasyon verimleri %85-92 olarak belirlenmiştir. Çalışma doğrultusunda A-549 ve MCF-7 hücre hatlarında gerçekleştirilen antikanser çalışmaları sonucu mikroalg ekstraktı yüklü nanopartiküllerin anti-kanser aktivite gösterdiği gözlemlenmiştir. Sonuç olarak elde edilen veriler doğrultusunda kullanılan kutup mikroalgi ekstraktı yüklü nanopartiküllerin antikarsinojenik ajan olarak biyoteknolojik ilaç kategorisinde değerlendirilme potansiyeli olduğu görülmüştür.

Anahtar Kelimeler: Kutup mikroalgleri, nanopartikül, anti-kanser aktivitesi, elektrospreyleme, enkapsülasyon

1
INTRODUCTION

Cancer is the second leading cause of death in the world (Siegel et al., 2023). The course of the disease and low survival rates affect the patients negatively. Although many diagnostic and treatment methods have been developed today, cancer treatment is still one of the leading research topics in the biotechnological field due to the late diagnosis of the disease, the type and size of the tumor, the patient's history, the non-specificity of the drug used for the tumor and its toxic properties (U. Anand et al., 2023; Karamitopoulou, 2019). In this context, treatments using natural products have attracted more attention in recent years (Huang et al., 2021). It has been observed that microalgae, which has been used frequently in the field of health biotechnology with the biotechnological developments in recent years, exhibit anti-cancer properties in many cancer types due to its rich biochemical content and bioactive properties (Abd El-Hack et al., 2019; Martínez Andrade et al., 2018). The effect of flavonoids, one of the contents of microalgae, on anticancer activity comes to the forefront in line with the researches. While microalgae show natural killer cell activity against cancer cells, they also activate the immune system. By this way, while preventing the growth of cancer cells, they also strengthen the body's defense system (Cichoński & Chrzanowski, 2022).

The aim of the current project is to encapsulate polar microalgae extracts obtained from two different polar microalgae species isolated from different regions of the poles by electrospraying method and to investigate the anticancer activity of the obtained potential biotechnological products to present an alternative treatment method with less side effects for cancer treatment. Studies on the anticancer activity of polar microalgae are limited in the literature, and this study will be a pioneer in the literature. The data obtained from different cancer types exposed to microalgal flavonoid extracts will guide many studies and will close many deficiencies in the literature. At the same time, detailed characterization of polar microalgae will be carried out.

1.1 Hallmarks of Cancer

Cancer disease has existed for centuries and is still a secondary cause of death worldwide (Md. M. Rahman et al., 2022). According to the latest data shown in Figure 1.1, approximately 19 million people get cancer, and 10 million people die from this disease, annually (Sung et al., 2021).

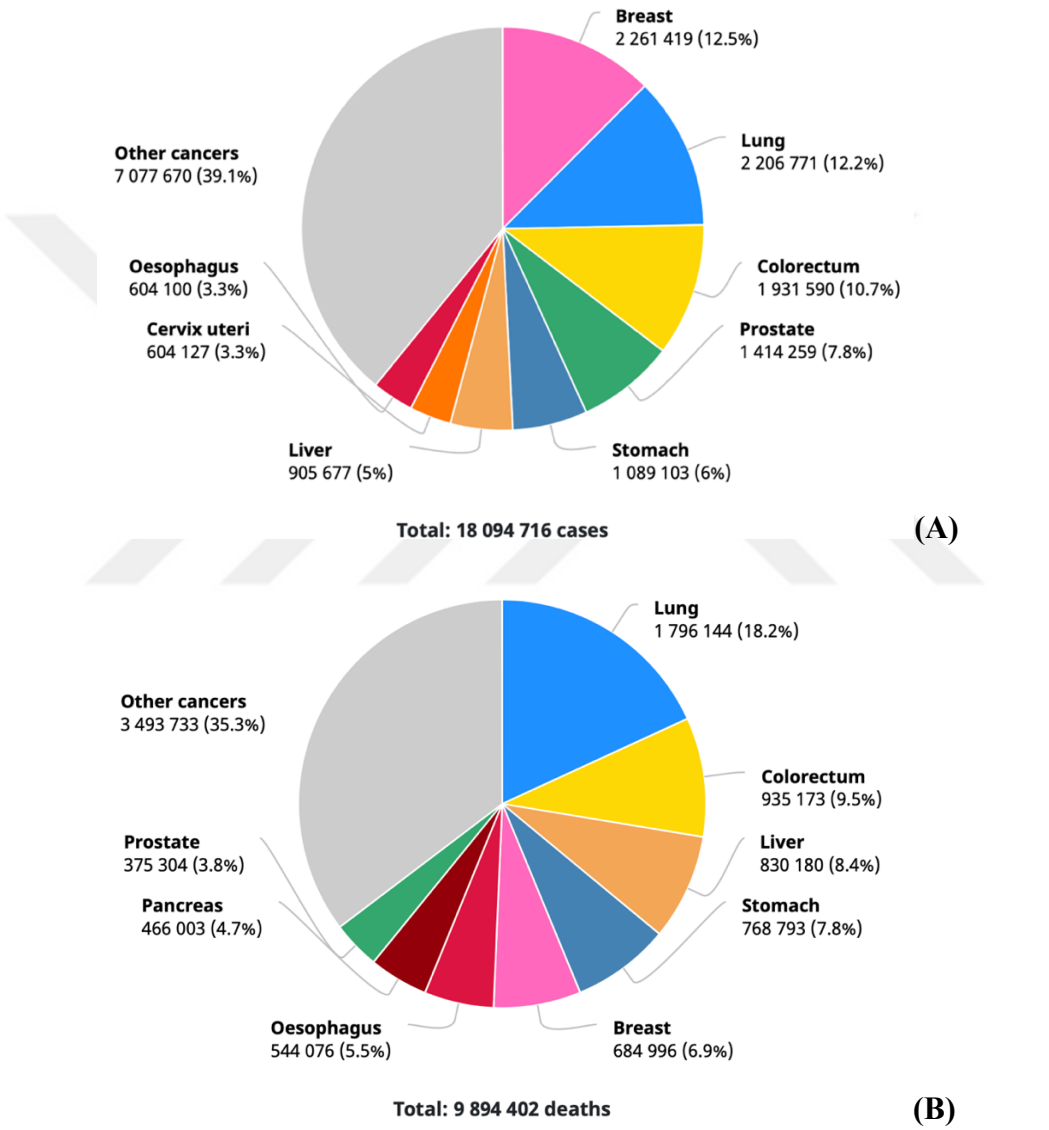


Figure 1.1 New cancer cases **(A)** and deaths **(B)** with both sexes worldwide in 2020

As can be seen from the graphs above, there are many types of cancer. Among them, breast and lung cancer are the most common and lethal types. While the incidence of lung cancer in men is 30%, the mortality rate is around 25%. In women, the most common type of cancer is breast cancer, while the incidence is 48%, while the mortality rate is close to 15%. Lung cancer, on the other hand, is one of the most common types in women, with an incidence of 15% and a mortality rate of 11%. Therefore, in this thesis, studies will be carried out on these two cancer types and comparative analyzes will be carried out.

As for the development of cancer disease, there are trillions of cells in our body, and every day senescent or damaged cells die to form new cells. This process repeats over and over again. However, if cells begin to divide uncontrollably during this cycle, cancer cells occur (A. K. Kashyap & Dubey, 2022; Sriharikrishnaa et al., 2023). Cancer is a disease in which some cells in the body develop uncontrollably or acquire mutations and then spread to other parts of the body in order to grow its original location when there is no longer an environment for growth. Exposure to carcinogens contributes to the development of cancer (Falzone et al., 2016; Smith et al., 2020).

However, not all of these cancer cells are harmful. Some cancer cells are referred to be benign tumors which neither invade surrounding normal tissue nor spread to distant body sites, remaining confined to their original location (Alzahrani et al., 2021; Cooper, 2000). Malignant tumors, on the other hand, grow as a result of uncontrolled cell division and accumulation, and tumor progression happens as a result of acquiring new additional gene mutations (Fouad & Aanei, 2017; Korgaonkar & Yadav, 2019). As a result of clonal selection, species that dominate in the tumor population are considered to be malignant tumors. They primarily invade surrounding tissues. After a while, when the environment and growth factors necessary for their growth are insufficient or there is no space for them to invade, angiogenesis takes place, and they enter the circulatory system by developing their own blood vessels. As a result, they spread to other parts of the body, a process known as metastasis (Figure 1.2) (Compton, 2020; Lambert et al., 2017).

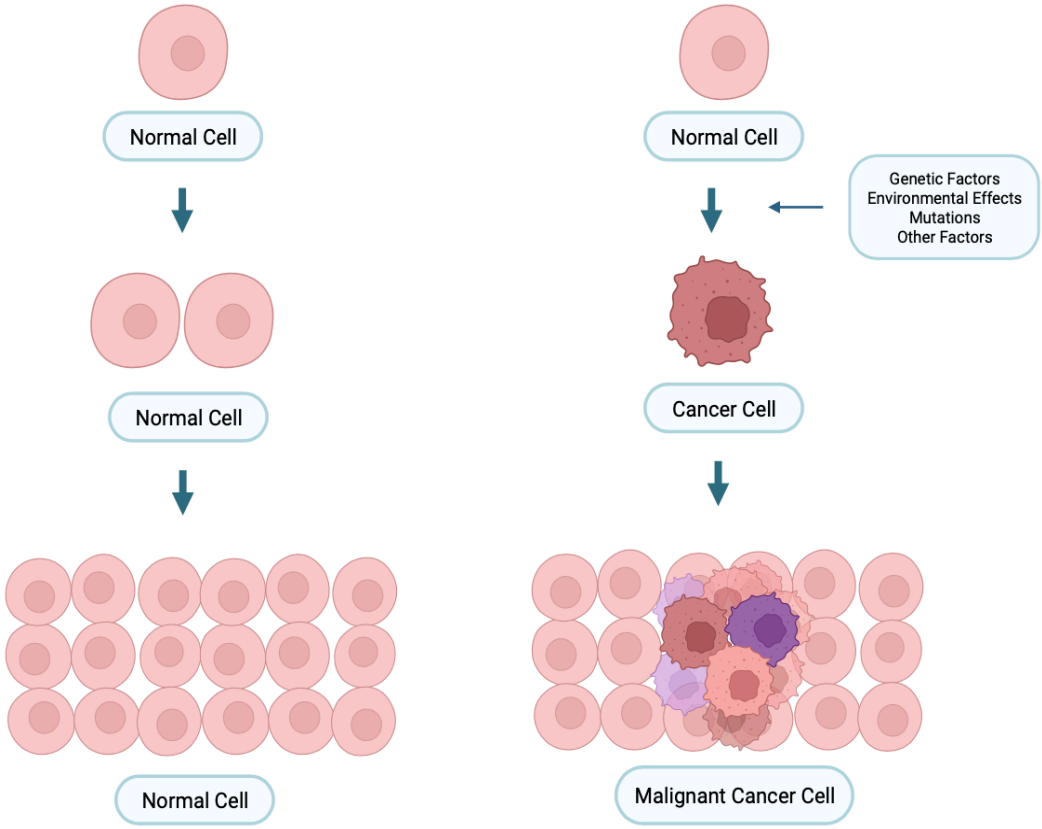


Figure 1.2 Stages of cancer cell development process

Cancer fundamentally has six hallmarks (Figure 1.3). These include resisting cell death, sustaining proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, and inducing angiogenesis (Hanahan & Weinberg, 2011). Epidemiology and progression of the disease, patient's condition, cancer type, environmental conditions, genetic factors can be shown as the reason for this lethal disease (P. Anand et al., 2008; Garssen, 2004).

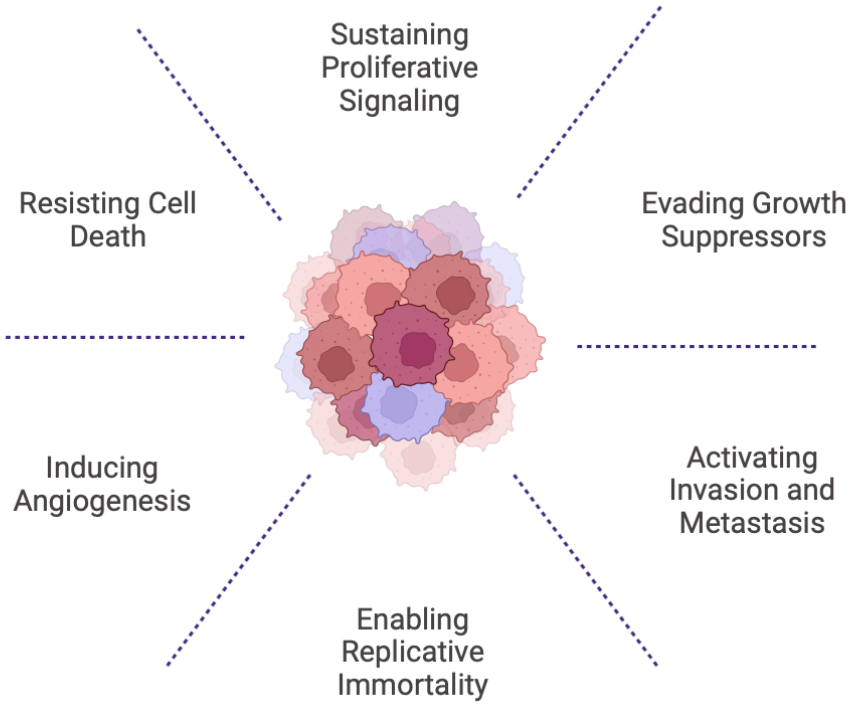


Figure 1.3 The six hallmarks of cancer

Over the past three decades, researchers have inquired about the regulation of the mechanisms of these hallmarks, gathering knowledge about genes and proteins and their function in the development of cancer cells and enhancing the prognosis of the disease (Elmore et al., 2021; Paul, 2020). However, there is still no definitive solution or treatment to this lethal disease (Hassanpour & Dehghani, 2017). The manner of therapy also varies depending on a variety of criteria, including the type of tumor, tumor stage, size, and location. Basically, depending on the complexity of the tumor, surgical removal, chemotherapeutic targeting, radiotherapy, hormonal therapy, immunotherapy, or smart drug use, which have become widespread in recent years, come to the fore in treatment methods. Unfortunately, many treatments fail to achieve expectations due to resource and diagnostic tool constraints, a lack of personalized treatment strategies, and the widely different genetic features of tumor populations (Debela et al., 2021; Pozzar et al., 2018). Furthermore, while chemical-based drugs used in traditional treatment procedures decelerate the course of the disease, due to their structure, these drugs damage both

healthy and cancerous cells and cause long-term challenges (Yildizhan et al., 2018). Patients may also develop a drug resistance mechanism over the course of their treatment at some point. Despite the fact that new generation drugs are emerging day by day, problems such as toxicity, tumor cell specificity, or resistance mechanisms still persist. For cancer patients, who are already going through a challenging process this creates an undesirable situation (Elmore et al., 2021; D. Kashyap et al., 2021). In order to prevent such problems, researchers search for alternative treatment methods with less side effects. In this context, it can be mentioned about biotechnological drugs obtained with natural products (Choudhari et al., 2020; Thomford et al., 2018); such as microalgae (Abd El-Hack et al., 2019; İnan, Mutlu, et al., 2023).

1.2 Features of Microalgae

One of the most prevalent primary producer species on earth, green algae are found in both freshwater and marine habitats (Leliaert et al., 2011; Yuan et al., 2020). Microalgae, which have significant applications in the field of biotechnology, have been shown to be present in a wide range of studies due to the their abundant content and the bioactive features (Garrido-Cardenas et al., 2018; Pulz & Gross, 2004). Due to these features, it is used in many fields such as medicine, pharmaceutics, health, cosmetics, energy sector, etc (K. M. Rahman, 2020; Rizwan et al., 2018). Furthermore, microalgae offer some appealing qualities for large-scale sustainable production, such as high biomass yields and easy conditions for growth. The medium is supplemented with the necessary components during cultivation to ensure that the microalgae grow effectively (Dolganyuk et al., 2020; Torres-Tiji et al., 2020). Many parameters such as temperature, light intensity, humidity, cultivation time, stirring, pH, medium content, microalgae species affect the growth of microalgae (Dolganyuk et al., 2020; Hossain & Mahlia, 2019).

Polar algae are species that have evolved to a wide variety of extreme conditions through a variety of different mechanisms. In recent years, studies on the adaptation strategies of polar microalgae to extreme conditions and the components obtained from polar microalgae have gained importance (İnan, 2023; Lauritano et al., 2020; Z. Zhang et al., 2019). Microalgae contain a variety of compounds that serve as defense molecules and protect them from extreme conditions (Hamidi et al., 2020; Lyon & Mock, 2014). To adapt to extreme environmental conditions, polar microbes change their structure and function, as well as their bioactive content (İnan, 2023; İnan, Mutlu, et al., 2023). Furthermore, there are few studies in the literature on microalgae isolated from polar regions, and these studies are mainly confined to species isolation and the effect of some parameters on microalgae growth.

In the thesis study, *Auxenochlorella pyrenoidosa* Ozcimen.001 *and Chlorella sorokiniana* Egemen.002 polar microalgae species which are Antarctic and Arctic microalgae species were used. *Chlorella sp.* microalgae have distinctive characteristics such as having a high nutritional composition, rapid growth in culture, resistance to extreme environmental conditions, less susceptible to

contamination, potential in biological activities, etc. (Yuan et al., 2020). It is critical to understand the morphology of microalgae species when conducting research in order to forecast their behavior and develop predictions for changing environments (Rendón-Castrillón et al., 2021). *Chlorella* sp. are green microalgae that are single-celled, non-motile, and range in diameter from 2 to 10 micrometers. *Chlorella* sp. possesses spherical or oval cell shape with a strong carbohydrate rich cell wall, which can be advantageous or disadvantageous during cultivation. There are also exterior and inner cell walls in some of species (Ievina & Romagnoli, 2020; M. A. Rodrigues & Bon, 2011; Yuan et al., 2020). Apart from that, they contain many organelles in the cell (Safi et al., 2014). The full lineage version of *Chlorella sp.* represented in table below (Schoch et al., 2020; Sexton & Lomas, 2018).

Table 1.1 Taxonomic classification of *Chlorella sp.* microalgae

Green Lineage of *Chlorella sp.* Microalgae	
Superkingdom	Eukaryota
Kingdom	Viridiplantae
Phylum	Chlorophyta
Class	Trebouxiophyceae
Order	Chlorellales
Family	Chlorellaceae
Genus	*Chlorella*

The green lineage, which marked the origin of the earliest oxygenic photosynthetic eukaryotes originated after endosymbiotic event almost 1 and 1.5 billion years ago (Leliaert et al., 2012). The taxonomy of *Chlorella* has evolved over time, and it is currently based on morphology, such as the solitary nature of the organism, the oval or spherical shape of the cells, etc. However, it is not that simple and straightforward to classify *Chlorella* sp. just by looking at its morphology alone, also it must be considered another parameter. The formation of new species and scientific inquiry

are both impacted by this current systematic classification (Bock et al., 2011; Ievina & Romagnoli, 2020; Krienitz et al., 2015).

Microalgae have been widely recognized in recent years due to their high bioactive content. It can be counted as biochemical content, as carbohydrate, lipid and protein contents, and bioactive content, as vitamin, pigment, phenolic contents (Andriopoulos et al., 2022; Surkatti & Al-Zuhair, 2018).

1.2.1 Flavonoids

Polyphenols are chemical compounds of the secondary metabolism, and flavonoids are hydroxylated phenolic substances that play an essential role in photosynthesizing cells (Karak, 2019). Flavonoids are natural pigments found in plant or microbial sources and correspond to a specific group of chemical components called natural phenolic compounds, which are synthesized as secondary metabolites (Devi et al., 2021; Imran et al., 2019; Mateos et al., 2020). Flavonoids are classified according to differences in the structure of the heterocyclic C ring shown in Figure 1.4 (Chaves et al., 2020; Chávez-González et al., 2020; Tzanova et al., 2020).

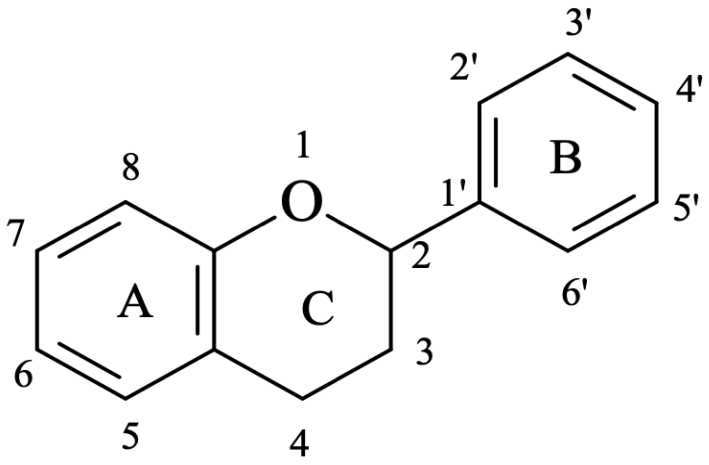
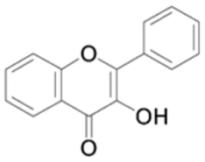
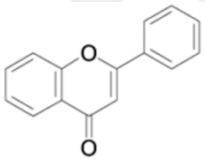
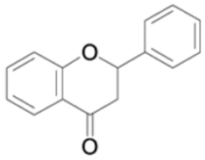
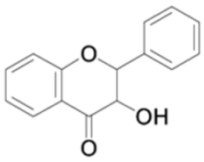
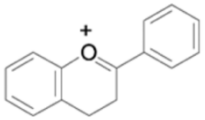
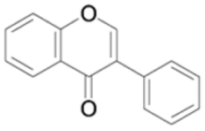


Figure 1.4 Basic structure of flavonoids (Tzanova et al., 2020)

Flavonoids have a potential role in promoting health and preventing chronic degenerative diseases (Dobrzynska et al., 2020; Karak, 2019). This is caused by the strong antioxidant, anti-inflammatory, and anti-cancer activities and bioactive properties of flavonoids. In this context, flavonoids have gained prominence in recent years in cancer research due to their ability to inhibit carcinogenesis and their use in the treatment of neurodegenerative diseases (Calderaro et al., 2022; Nijveldt et al., 2001; Ruiz-Cruz et al., 2017). As secondary metabolites, microalgae also contain flavonoids. The content of microalgal flavonoids has been determined by several research in the literature (Baviskar & Khandelwal, 2015; Goiris et al., 2014). Studies on flavonoids obtained from plant or algae species have been found to show anticancer activity, yet the studies using polar microalgae are limited (Ekalu & Habila, 2020; Ferdous & Balia Yusof, 2021). Flavonoids regulate certain signal pathways, inducing cell proliferation, apoptosis mechanism, MAP-kinase pathway (Ramesh et al., 2021). Its effects on apoptosis, angiogenesis, and metastasis pathways have been proven by different studies (Bonferoni et al., 2017, 2017; Imran et al., 2019). In some studies, it has been observed that it reduces the growth of tumor cells (Wali et al., 2019). Subclass, chemical structure and compound examples of different types of flavonoids are listed in table below (Ayaz et al., 2019; Chaves et al., 2020; Devi et al., 2021; Kawser Hossain et al., 2016; Z. Wang et al., 2013).

Table 1.2 Subclass, chemical structure and compound examples of different types of flavonoids

Subclass of Flavonoid	Structure	Examples of Flavonoid Compounds
Flavonol		○ Quercetin ○ Kaempferol ○ Myricetin ○ Tamarixetin
Flavone		○ Chrysin ○ Apigenin ○ Rutin ○ Luteolin
Flavonone		○ Naringin ○ Taxifolin ○ Hesperidin ○ Fisetin
Flavanol		○ Catechin ○ Epicatechin ○ Epigallocatechin
Anthocyanidin		○ Apigenidin ○ Delphinidin ○ Malvidin Peonidin
Isoflavone		○ Genistein ○ Daidzein

1.3 Encapsulation Processes

Problems arise such as the easy degradation of most of the bioactive substances, such as antioxidants, due to environmental effects, during the use phase after the acquisition of bioactive substances. Studies that will prevent the bioactive material from degradation and enhance its adsorption properties are therefore required. In this context, encapsulation is made for many purposes such as increasing the shelf life and increasing the nutritional value of the products (Martínez-Ballesta et al., 2018; Piñón-Balderrama et al., 2020; Zabot et al., 2022). One of the most common methods of encapsulating is the fabrication of nanoparticles loaded with bioactive compounds. Nanoparticles can have the shape of nanocapsules or nanospheres, depending on the manufacturing method (Aghababai Beni & Jabbari, 2022). In encapsulation, the bioactive substance or essential oil is preserved by enclosing it in a coating material in the core. Coating material may consist of materials such as lipid, metal (gold, silver, zinc etc.), protein (gelatin, collagen, casein, etc.), carbohydrates (cellulose, starch, alginate, etc.), natural polymers (alginate, chitosan, agarose, etc.) or artificial polymers (PEG, PVA, PLGA, etc.) (Ayyaril et al., 2023; Pateiro et al., 2021; Primožič et al., 2021). For the encapsulation process, there are both physical and chemical approaches. The most often utilized techniques among these are ionic gelation, spray drying, emulsion, and electrospinning/electrospraying (Figure 1.5) (Ayyaril et al., 2023; Balla et al., 2022; Peng et al., 2020). The use of electrospinning/electrospraying for producing nanoparticles allows for the preservation of bioactive components and more effective targeting. Encapsulation also reduces side effects and toxicity (Brezániová et al., 2018). The smaller the size of the nanoparticle, the higher the rate of action that it can have on cancerous cells. This is because it is easier for the nanoparticle to enter cells and the circulatory system (Mitchell et al., 2021). Numerous factors, including temperature, the characteristics of the core material, viscosity, surface tension, and voltage, have an impact on this process (Drosou et al., 2017).

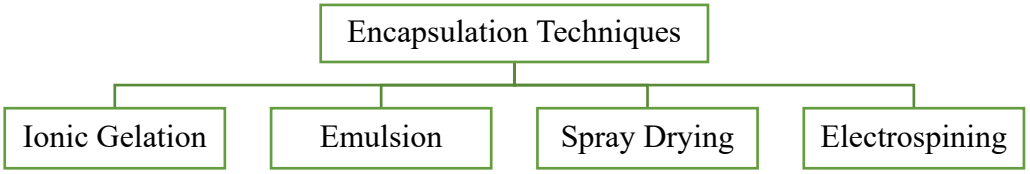
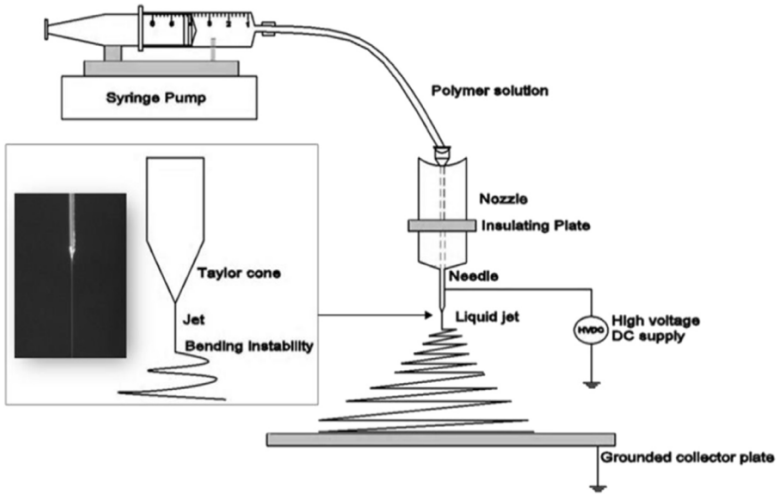


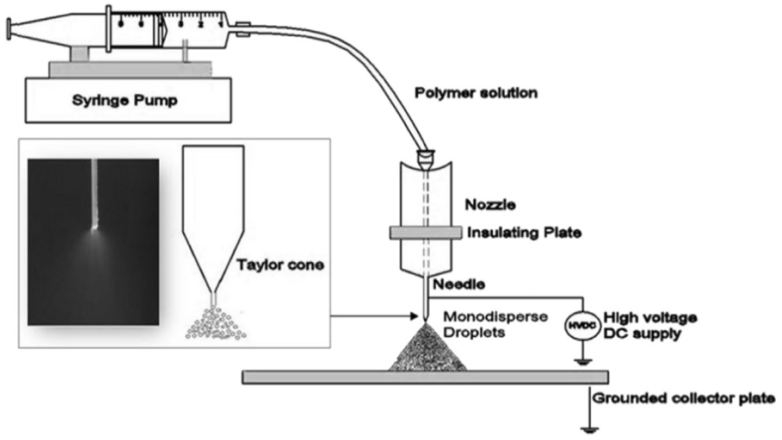
Figure 1.5 Main encapsulation techniques

Electrospinning is a very convenient and useful approach that has been enhanced over time with developments. It is now widely used as a result of the developments that have taken place in biotechnology and nanotechnology (Kumar Sharma & Rachel James, 2023; Xue et al., 2019). The electrospining process is influenced by parameters including solution-related concentration, polymer molecular weight, viscosity, and surface charge; however, many other variables, including voltage, feed rate/flow rate, type to collector distance, and collector type, are also used as process-related parameters (Bhardwaj & Kundu, 2010; Moreira et al., 2021). Nanoparticles can be obtained from polymer solutions or biological materials as a result of this approach (Stojanov & Berlec, 2020). The electrospining process is utilized in a wide range of fields, such as food, cosmetics, pharmacuetic, medicine, and biomedical applications, as well as the energy sector, filtration processes, textiles sector, and sensor construction (Kumar Sharma & Rachel James, 2023).

Electrospinning and electrospraying are related and innovative electrohydrodynamic methods in which a suspension or solution can be spun or sprayed by applying a high direct current voltage to form fibers or particles, respectively as shown in Figure 1.6 (Khoshnoudi-Nia et al., 2020; Li et al., 2021). Basically, this method produces either nanofibers or nanoparticles by spinning or spraying the polymer solution at high voltage in an electric field. An electrospinning and electrospraying setup typically consist of four basic parts: a high-voltage source, a needle or capillary, a syringe pump, and a collector, which can be either a flat plate or a revolving drum. In electrospinning, a high voltage potential induces free charges in the polymer solution in the capillary. The process of electrospraying, on the other hand, involves the electrical atomization of liquids (Anu Bhushani & Anandharamakrishnan, 2014).



(A)



(B)

Figure 1.6 The schematic represantation of electrospinning **(A)** and electrospraying **(B)** processes (Anu Bhushani & Anandharamakrishnan, 2014)

1.4 Microalgae in Cancer Research

Thanks to their bioactive substance content, microalgae show wide bioactivity including antioxidant, anti-inflammatory, anti-viral and anti-cancer properties, and it is known that they show especially high anti-cancer activity (Patil et al., 2022; Saide et al., 2021). Cancer diagnosis and treatment methods are insufficient due to their characteristics such as uncontrolled division of cancer cells, different behaviors in different tissues or regions (Alberts et al., 2002; Chakraborty & Rahman, 2012; H. Wang et al., 2019), invading neighboring tissues or metastasizing through the blood/lymphatic system (Leyva-González et al., 2021), and having a heterogeneous structure (Pucci et al., 2019). Problems such as the wrong region targeting of traditional methods or the toxicity they cause in normal and healthy cells, and problems such as drug resistance affect patients more negatively (Fraguas-Sánchez et al., 2020; A. Lin et al., 2019). In order to prevent such problems, the search for alternative treatments with fewer side effects has begun. In this context, microalgae stand out due to their rich bioactive components (Abd El-Hack et al., 2019). As a result of the research, it has been suggested that microalgae exhibit anti-cancer properties in many cancer types (Ferdous & Balia Yusof, 2021; İnan et al., 2021; İnan, Mutlu, et al., 2023). Microalgae, due to their rich content, show an attitude towards reducing the viability of cancerous cells, while at the same time they provide the activation of the immune system. By this way, while preventing cancer cell growth in the body, they also improve the body's defense mechanism (Abd El-Hack et al., 2019). Table 1.3 gives a summary of some of the investigations that have been conducted on the anticancer properties of microalgae and polar microalgae.

Table 1.3 Summary of microalgae used in cancer research

B.braunii and *M. aeruginosa*	AGS gastric cancer cells PC-3 human prostate cancer cell line SHSY-5Y neuroblastoma cell line L929 fibroblast cells	(İnan et al., 2021)
C. pyrenoidosa	HepG2 liver cancer cells	(X. Wang & Zhang, 2013)
C. protothecoides and *N. oculata*	A-172 human brain glioblastoma cells HCT-116 colon colorectal carcinoma cells	(Karakaş et al., 2019)
C.ovalis and *N. oculata*	HL-60 human promyelocytic leukemia cell A549 human lung carcinoma cells B16F10 mouse melanoma cell line	(Samarakoon et al., 2013)
C. danica	A375 melanoma cancer cells A549 human lung cancer cells HepG2 liver cancer cells HT29 colorectal adenocarcinoma cells	(Kilari & Balakrishnan, 2023)
C. vulgaris	AGS gastric cancer cells	(Sheih et al., 2010)
C. vulgaris	MCF-7 breats cancer cells	(Skjånes et al., 2021)
C.sorokiniana	*A549 and CL1-5 human lung adenocarcinoma cells*	(P.-Y. Lin et al., 2017)

Table 1.3 Summary of microalgae used in cancer research (continued)

C. sorokiniana and *Scenedesmus* sp.	L5178Y-R lymphoma cell line and normal lymphocyte	(Reyna-Martinez et al., 2018)
C. pyrenoidosa FACHB-9, Scenedesmus sp. and Chlorococcum sp.	HCT116 human colon cancer cells HCT8 human colon cancer cells	(J. Zhang et al., 2019)
N. oculata	MDA-MB-231 human breast cancer cells	(Goh et al., 2014)
D. salina	MCF-7 breats cancer cells	(Singh et al., 2019)
D. salina	PC-3 human prostate cancer cell line	(Jayappriyan et al., 2013)
H.pluvialis	MDA-MB-231 human breast cancer cells	(Alateyah et al., 2022)
N. oculata	MDA-MB-231 human breast cancer cells	(Wali et al., 2020)
L. papillosa	MCF-7 human breast cancer cells	(Elia et al., 2020)
S. platensis	Lung metastasis of breast cancer	(Zhong et al., 2020)
S. maxima, C. salina, N. oceanica, and N. oculata	MCF-7 human breast cancer	(Elkhateeb et al., 2020)

Table 1.3 Summary of microalgae used in cancer research (continued)

S. platensis	HepG2 liver cancer cells	(Prabakaran et al., 2020)
Picochlorum sp.	MDA-MB-231 human breast cancer cells MCF-7 human breast cancer cells Hep-G2 human liver cancer cells A549 human lung cancer cells HDF human dermal fibroblast cells	(Abolhasani et al., 2018)
Tribonema sp	HepG2 liver cancer cells	(Chen et al., 2019)
Botryidiopsidaceae sp.	A375 melanoma cancer cells Hs578T breast cells HeLa cervical carcinoma cells HaCaT non-tumorigenic keratinocyte cells	(Suh, Kim, et al., 2017)
C. variabilis and *A. pyrenoidosa*	AGS gastric cancer cells PC-3 human prostate cancer cell line	**(**İnan, Mutlu, et al., 2023)
Chloromonas sp.	A375 melanoma cancer cells Hs578T breast cells HeLa cervical carcinoma cells HaCaT non-tumorigenic keratinocyte cells	(Suh, Yang, et al., 2017)
Chloromonas reticulata	*HCT116 human colon cancer cells*	(Suh et al., 2019)
Micractinium sp.	*HCT116 human colon cancer cells*	(Suh et al., 2018)

2
MATERIALS AND METHODS

2.1 Cultivation of Polar Microalgae

In the thesis study, *Auxenochlorella pyrenoidosa* Ozcimen.001 (CP) *and Chlorella sorokiniana* Egemen.002 (CS) microalgae species which are Antarctic and Arctic microalgae species isolated and identified at YTU-Algal Biotechnology and Bioprocess Laboratory are used. This microalgae species is collected under the auspices of the Presidency and under the responsibility of the Ministry of Industry and Technology; with the 3rd National Antarctic Science Expedition (TAEIII) held between 29 January-7 March 2019 and the first Turkish Arctic Science Expedition (TASE-I) carried out by ITU Polar Research Application and Research Center (PolReC) between 11-26 July 2019. The samples were found to be suitable for cultivation in the laboratory due to their high adaptability. In this context both solid and liquid media were used for cultivation, and two different polar types of microalgae species were grown with those conditions.

Microalgae were first grown in Bold's Basal Medium (BBM) medium containing 15 gr agar to create a stock culture. In this direction, the chemicals prepared in the ratios indicated in Table 2.1 and Table 2.2 were added to glass schott bottles respectively, and the medium was prepared by completing with distilled water until the total volume was 1000 mL. The prepared medium was autoclaved at 121°C and 1 atm of pressure. After the autoclave procedure was completed, the warm medium was poured into petri dishes and allowed to solidify. The relevant polar microalgae cultures were inoculated with using a spreader. After parafilming around the petri dishes, they were placed in a Kerman brand incubator with continuous lighting for 10 days under the required conditions (Figure 2.1). Then stock culture was obtained by washing process.

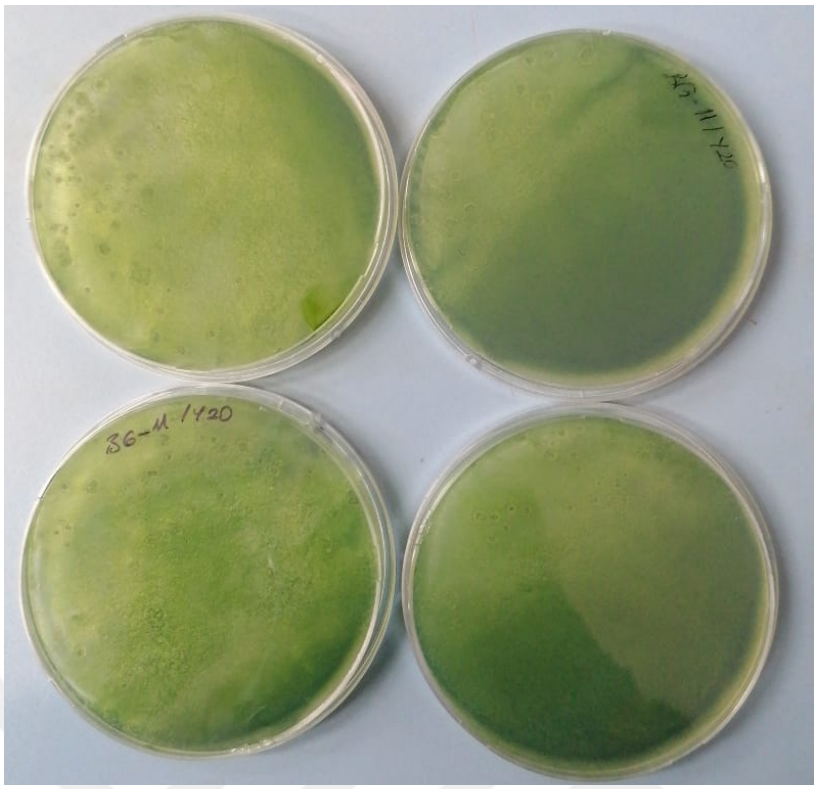


Figure 2.1 Microalgae inoculation in solid agar media with BBM medium

Microalgae cultures are cultivated in liquid cultures in 150 mL flasks after the stock culture has been obtained. For preparation of liquid cultures 10% microalgae stocks solution, 2% BBM broth medium, which is prepared with the chemicals specified in Table 2.1 and Table 2.2, was added into flask, then this solution completing with distilled water until the total volume was 150 mL. In Kerman brand incubator, prepared microalgae liquid cultures were cultivated for 15 days at 150 rpm stirring speed and 25°C with continuous lighting (Figure 2.2). Using the PG 60 Instrument UV-Vis Spectrophotometer, the absorbance of microalgae at a wavelength of 680 nm was measured in order to monitoring their growth. Pure water was used as the 'blank' for this measurement. The daily absorbance data was used to obtain the growth kinetics of both microalgae species. These stages were carried out in triplicate. The specific growth rate (μ) and doubling time (t_d) values of microalgae were estimated based on the data collected from the growth kinetics curve (Christwardana et al., 2022; Saleem et al., 2022). These values were calculations with the help of equation 2.1 and 2.2 and growth kinetics curve.



(A) **(B)**

Figure 2.2 Liquid CP polar microalgae culture **(A)** and CS polar microalgae culture **(B)**

$$\text{Specific growth rate } (\mu) = \frac{(\ln OD_t - \ln OD_0)}{\Delta t} \quad (2.1)$$

OD_0 = Optical density value measured at beginning time

OD_t = Optical density value measured at time t

$$\text{Doubling time } (t_d) = \frac{ln2}{\mu} \quad (2.2)$$

Table 2.1 Chemicals used in BBM medium

Volume(mL)	Stock Solution	Concentration (g/400 mL H_2O)	Final Concentration (g/L)
10	$NaNO_3$	10.0	0.25
10	$CaCl_2.2H_2O$	1.0	0.025
10	$MgSO_4.7H_2O$	3.0	0.075
10	K_2HPO_4	3.0	0.075
10	KH_2PO_4	7.0	0.175
10	NaCI	1.0	0.025
1	H_3BO_3	4.56	0.0114
1	Na_2EDTA	20.0	0.05
1	KOH	12.4	0.031
1	$FeSO_4.7H_2O$	1.992	0.005
1	Trace Element Solution		

Table 2.2 Chemical contents of trace element solution

Compound	Quantity (g/L)
$ZnSO_4.7H_20$	8.82
$MnCl_2.4H_2O$	1.44
$CuSO_4.5H_2O$	1.57
MoO_3	0.71
$Co(NO_3).6H_2O$	0.49

Furthermore, the microalgal biomass obtained from the production was harvested by centrifugation at 8000 rpm for 10 minutes using a PG Instruments T60 model refrigerated centrifuge. After centrifugation, the resultant upper liquid supernatant phase was poured, and the solid microalgal biomass in the pellet phase was dried in a Binder brand oven set to 60°C. The solid microalgal biomass produced after drying process was used in biochemical content analyses and anticancer research.

2.1.1 Determination of Carbohydrate Content

The total carbohydrate content of the polar microalgae species utilized in this study was estimated using the phenol-sulfuric acid technique. Glucose was employed as the standard for analysis in this procedure. Firstly, phenol solution was prepared as 80% (w/w) concentration. Glucose solutions were prepared at the rates indicated in the Table 2.3 in glass tubes. Then 50 μL of the prepared phenol solution and 5 mL of H_2SO_4 were added to the prepared glucose solutions in fume hood, respectively. After incubating the solutions for 10 minutes at room temperature, their absorbance at 490 nm was measured with a PG Instruments T60 model UV spectrophotometer to produce a calibration curve. To determine the carbohydrate content

of polar microalgae species, 3-5 mg of dry microalgae biomass was measured using a precision scale. Following that, 2 mL of distilled water, 50 μL of phenol solution and 5 mL of H_2SO_4 solution were added to a glass tube, respectively under a fume hood. The glass tubes were then incubated for 10 minutes at room temperature. At the end of the incubation, the absorbance of the samples was measured at 490 nm in the PG Instruments T60 model UV spectrophotometer, and the carbohydrate content of microalgae cultures was estimated using the calibration curve (Tarhan et al., 2020).

Table 2.3 Standard glucose concentration values

	µg glucose/mL					
	0	20	40	60	80	100
Glucose stock solution (mL)	0	0.2	0.4	0.6	0.8	1.0
Distilled water (mL)	2	1.8	1.6	1.4	1.2	1.0



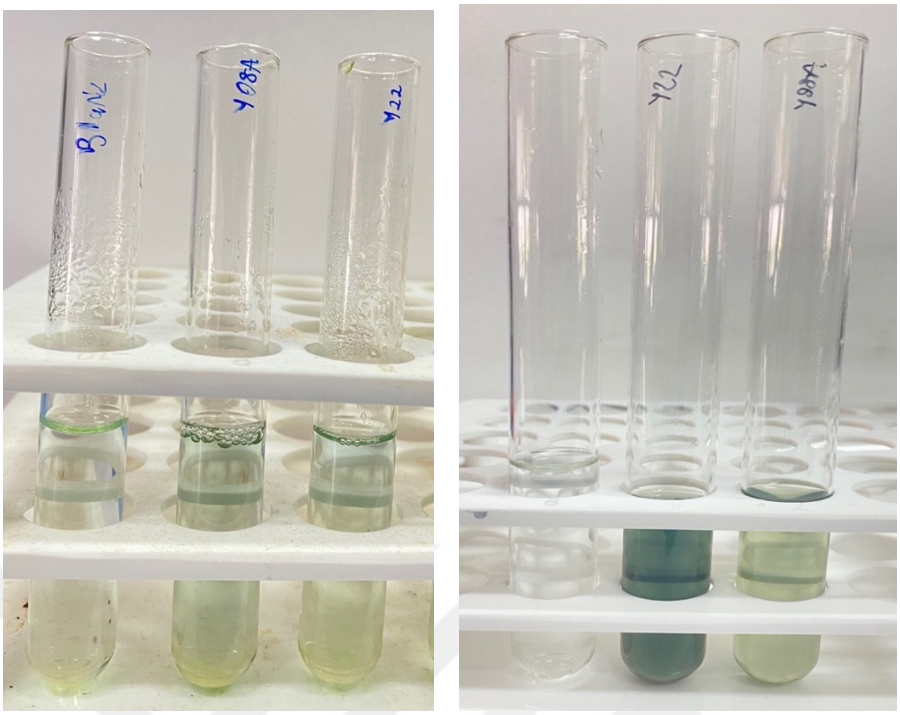
Figure 2.3 Carbohydrate content determination results

2.1.2 Determination of Protein Content

The Modified Lowry Method was used for determining amounts of protein in the polar microalgae species employed in this study (Lowry et al., 1951; Redmile-Gordon et al., 2013; Tarhan et al., 2020). The BSA solution was employed as a standard for analysis in this procedure. To prepare the standard solutions, distilled water and BSA in the ratios in the Table 2.4 are added to the glass tubes. Then 0.5 mL of 1 N NaOH solution was added to the solutions made in glass tubes and kept in an Alex Machine Ultrasonic Cleaner brand water bath set to 80°C for 5 minutes. Afterwards, the tubes were left to cool down and, 2.5 mL of Reagent C solution and 0.5 mL of Reagent D solution were added to the tubes, respectively. The tubes were then vortexed and left to incubate in the dark for 30 minutes at room temperature. The calibration curve was obtained at the end of the incubation process by measuring the absorbance values of solutions at 750 nm in the PG Instruments T60 model UV spectrophotometer. To determine the protein content of polar microalgae, 3-5 mg of dried polar microalgae biomass was weighed using a precision balance, then 0.5 mL of distilled water and 0.5 mL of 1 N NaOH solution to the dry biomass in the glass tube, respectively and kept in an Alex Machine Ultrasonic Cleaner brand water bath set to 80°C for 5 minutes. Afterwards, the tubes were left to cool down and, 2.5 mL of Reagent C solution and 0.5 mL of Reagent D solution were added to the tubes, respectively. The tubes were then vortexed and left to incubate in the dark for 30 minutes at room temperature. The calibration curve was obtained at the end of the incubation process by measuring the absorbance values of solutions at 750 nm in the PG Instruments T60 model UV spectrophotometer.

Preparation of reagents and solutions used in protein analysis:

- Reagent A solution: 5% Na_2CO_3 solution was prepared in distilled water.
- Reagent B solution: 0.5% $CuSO_4.5H_2O$ in 1% K-Na-tartarate was prepared.
- Reagent C solution: It was prepared by mixing 50 mL of Reagent A solution with 2 mL of Reagent B solution.
- Reagent D solution: 1 N Folin-Ciocalteau solution was prepared by diluting 2 N Folin reagent with distilled water at 1:1 ratio.



(A) **(B)**

Figure 2.4 Samples before **(A)** and after **(B)** protein content analysis

Table 2.4 Standard and reagents of the Modified Lowry Method

	0	**20**	**40**	**60**	**80**	**100**	**Sample**
BSA stock solution (mL)	0	0.2	0.4	0.6	0.8	1.0	-
Distilled water (mL)	2	1.8	1.6	1.4	1.2	1.0	0.5
1 N NaOH (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Reagent C (mL)	2.5	2.5	2.5	2.5	2.5	2.5	2.5
Reagent D (mL)	0.5	0.5	0.5	0.5	0.5	0.5	0.5

2.1.3 Determination of Pigment Contents

To determine the amounts of β-carotene and chlorophyll-α, which are the pigment content of the polar microalgae species, 5 mL of liquid polar microalgae culture was taken into the falcon and precipitated using a PG Instruments T60 model refrigerated centrifuge at 8000 rpm for 10 minutes at 4°C. The supernatant phase at the top was then drained off, and 5 mL of acetone solution was added to the pellet at the bottom. Falcons were homogenized for 10 minutes in an Alex Machine Ultrasonic Cleaner brand ultrasonication-assisted water bath set to 60°C. The solution was then stirred for 15 seconds using a vortex to obtain a homogenous consistency. It was then precipitated again at 8000 rpm for 10 minutes at 4°C using a PG Instruments T60 model refrigerated centrifuge device, which was causing chloroplast fragmentation and the pigments in the microalgae were transferred to the solvent. The PG 60 Instrument UV-Vis Spectrophotometer was used to measure the absorbance of the upper liquid supernatant phase after centrifugation at 475 nm and 666 nm wavelengths. The quantity of β-carotene and chlorophyll- α from the absorbance data obtained through the equation 2.3 and 2.4 (Macías-Sánchez et al., 2005; D. B. Rodrigues et al., 2015; Soares et al., 2016).

$$\beta - \text{carotene } (\mu\text{g/mL}) \;=\; 4.5 \text{ x } OD_{475} \qquad (2.3)$$

$$\text{Chlorophyll} - \alpha \; (\mu\text{g/mL}) \;=\; 13.9 \text{ x } OD_{666} \qquad (2.4)$$

OD_{475} = Absorbance value measured at 475 nm

OD_{666} = Absorbance value measured at 666 nm

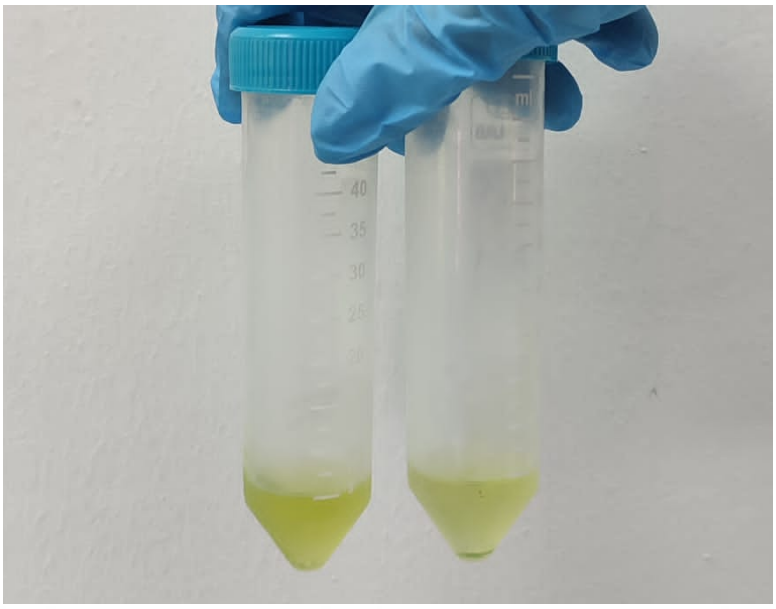


Figure 2.5 The samples for pigment analysis

2.1.4 Proximate Analysis

As a result of this analysis, the moisture content, volatile matter content, fixed carbon content, and ash content of microalgae species were determined using ASTM standards (García et al., 2013). After drying the raw materials in a Binder brand oven and storing them in a desiccator to maintain moisture balance, proximate analyses were performed in a TA Instrument brand SDT Q600 model thermogravimetric analyzer. In the analysis, the samples were heated at 110°C at a rate of 20°C/min in a nitrogen environment, then held at this temperature for 30 minutes to ensure that the moisture in the microalgal biomass was fully eliminated from the structure. The device was then heated to 600°C in a nitrogen environment at a rate of 40°C/min for 10 minutes after reaching 600°C. At this point, the volatile compounds in the sample were eliminated from the structure. Following this stage, the nitrogen gas was turned off and replaced with dry air, and the mass was held at this temperature in a dry air environment until it stabilized. At the end of this step, the residual part is ash, and the part that goes away from the structure is fixed carbon. The fixed carbon content was calculated by percentage of volatile matter and the percentage of ash (García et al., 2012; Koçer & Özçimen, 2021). The elemental compositions of polar microalgae were determined using the data obtained and the formulae provided below (Nhuchhen, 2016).

$$C = -35.9972 + 0.7698VM + 1.3269FC - 0.3250ASH \quad (2.5)$$

$$H = 55.3678 - 0.4830VM - 0.5319FC - 0.5600ASH \quad (2.6)$$

$$O = 223.6805 - 1.7226VM - 2.2296FC - 2.2463ASH \quad (2.7)$$

$$N = 100 - (C + H + O + Ash) \quad (2.8)$$

2.1.5 Fourier Transform Infrared Spectroscopy (FTIR)

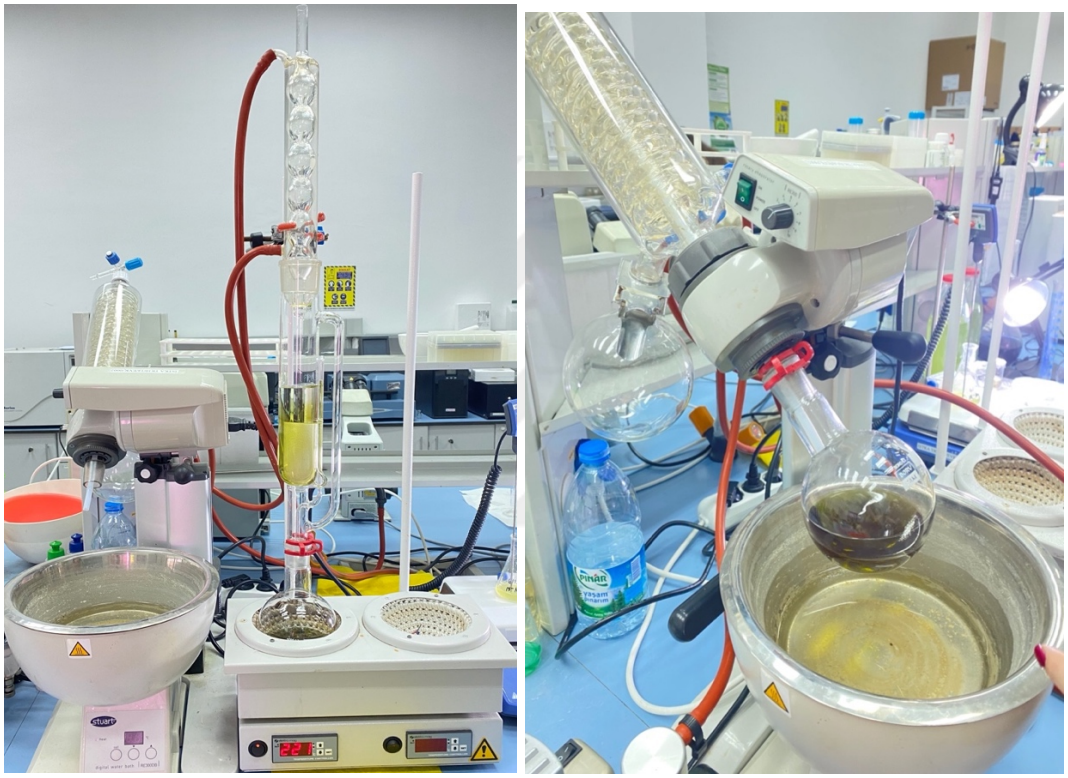
The functional groups of microalgae species were determined using a Bruker brand FTIR device (Figure 2.6). The ATR technique was used to determine the spectra of the microalgae samples utilized in the study at a resolution of of 4 cm^{-1} in the range of 500-4000 cm^{-1} (İnan & Özçimen, 2021; Kiran Kumar et al., 2018).



Figure 2.6 Bruker brand FTIR device

2.2 Extraction Process of Polar Microalgae

To obtain the extract from the polar microalgae species used in this study, the Soxhlet extraction method was performed. The dry microalgae biomass was weighed by taring the filter paper with precision scales and carefully placing it in the cartridge, which was then carefully placed in the Soxhlet apparatus. The flask was filled with 300 mL of 100% ethanol solvent, and the Soxhlet apparatus was attached by inserting the flask into the heater in the Soxhlet device (Figure 2.7).



(A) **(B)**

Figure 2.7 Extraction of polar microalgal extracts in the Soxhlet device **(A)** and evaporating the solvent with the rotary vacuum evaporator device **(B)**

The samples were extracted for three hours, and the operation was repeated three times. The flask is then placed in the rotary vacuum evaporator, and the ethanol in the extract is evaporated. The resultant extracts were weighed, and samples were collected in amber glass tubes to prevent oil oxidation. To evaporate the residual ethanol in the microalgae extract produced after extraction, it was placed in a

Bender brand oven set to 78°C. In this manner, ethanol was evaporated out of the amber bottles. The samples were kept in glass amber bottles (Figure 2.8) in a dark atmosphere by parafilming the bottles' caps (Zaini et al., 2022).



Figure 2.8 Polar microalgae extract after drying process

2.2.1 Determination of Lipid Content

To determine the total amount of lipid in the polar microalgae species utilized in the study, the raw weight of dry microalgae biomass was weighed using precision scales, as well as the total weights with filter paper. Following that, the extraction process was carried out using the Soxhlet method, as stated in the preceding section, and the samples were extracted. The acquired microalgae extracts were placed in a Binder brand oven set at 78°C to evaporate the ethanol in the extract produced after extraction (Figure 2.9). The amount of lipid obtained by weighing was determined using the equation 2.9 (Bligh & Dyer, n.d.; El-Bilawy et al., 2022).

$$\%\ Oil\ Content = \frac{Initial\ Weight - Final\ Weight}{DW\ of\ biomass}\ x\ 100 \qquad (2.9)$$



Figure 2.9 Sample before lipid content analysis

2.2.2 Gas Chromotography (GC) Analysis

To assess the content of microalgae extract, a YL Instruments 6100 GC brand gas chromatography device was used (Figure 2.10). The gas chromatography device incorporates a flame ionization detector (FID) and a 30 m x 0.32 mm x 0.25 m ZB-FFAP column. FID devices detect ions formed during the combustion of organic compounds with a hydrogen flame. It determines the amount of organic matter in the sample as well as the quantity of ions produced.

The column temperature program will begin at 50°C and gradually rise to 175°C at a rate of 15°C/min until reaching 230°C at a rate of 5°C/min. The flow rate was set at 1.8 mL/min, and the injector temperature was 230°C. The detector temperature is adjusted to 280°C. The carrier gas was hydrogen gas at a rate of 2 mL/min of hydrogen (99.9%). As an internal standard, methyl heptadeconate (C17:0) was employed, and the polar microalgae extract samples were combined with methyl heptadeconate and n-heptane to prepare them for gas chromatography (El-fayoumy et al., 2021; İnan et al., 2021).



Figure 2.10 YL Instruments 6100 GC brand device

2.2.3 Total Antioxidant Activity of Polar Microalgae Extracts

The total antioxidant activity of polar microalgae extracts utilized in the study was evaluated using the 1, 1-diphenyl-2-picryl hydrazil (DPPH) free radical scavenging technique. The process is based on bleaching the DPPH solution, which is normally purple in color but begins to change color due to the proton or electron donating ability of microalgae extracts. The decrease in absorbance in the reaction mixture indicates a significant level of free radical scavenging activity. To begin the experiment, 10 mg of 1, 1-diphenyl-2-picryl hydrazil free radical is weighed, and 300 mL of methanol is added to it, and it is stirred in 500 mL flasks for 1 hour in a magnetic stirrer set at 300 rpm, but flasks are wrapped in aluminum foil to prevent light and deterioration. The samples were prepared according to Table 2.5 values at concentrations of 100-1000 µg/mL with a total volume of 4.5 mL, and the blank was pure methanol.

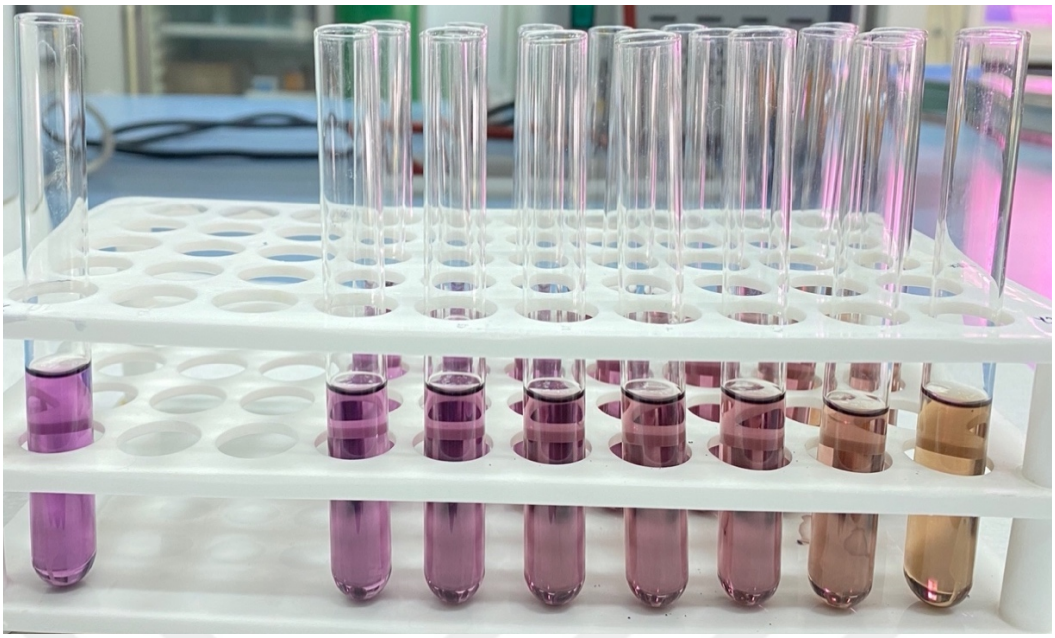


Figure 2.11 The solutions at different concentrations for DPPH analysis

The prepared samples were placed in a dark environment, and absorbance measurements were obtained at 517 nm wavelength using a PG 60 Instrument UV-Vis Spectrophotometer instrument at 30, 60, and 90 minutes. The following formula was used to determine the DPPH radical removal activity in accordance with the data acquired at end of the experiment (Monteiro et al., 2020; Nezafatian et al., 2023).

$$DPPH\ Deradicalization\ Activity\ (\%) = \frac{(OD_0 - OD_1)}{OD}\ x\ 100 \qquad (2.10)$$

OD_0: Absorbance value of the control

OD_1: Absorbance value of the sample or standard

Table 2.5 DPPH protocol for antioxidant activity

	Blank	**Control**	**100**	**250**	**300**	**350**	**500**	**1000**
Sample (μL)	-	-	100	250	300	350	500	1000
Methanol (μL)	4500	1500	1400	1250	1200	1150	1000	500
DPPH dye (μL)	-	3000	3000	3000	3000	3000	3000	3000

2.2.4 Determination of Total Phenolic Content (TPC)

Total Phenolic Content Determination (TPC) technique was utilized to determine the total phenolic contents of the polar microalgae extracts used in the study. In order to accomplish this, 5 mL of 0.2 N foline solution diluted with distilled water was added to a 1 mL sample in a glass tube and homogenized with a vortex. Then, 4 mL of a sodium carbonate (Na_2CO_3) solution containing 75 g/L was added to the mixture. As a control, 5 mL of 0.2 N folin solution was added to 1 mL of distilled water in a glass tube and vortexed. Following that, 4 mL of a 75 g/L Sodium Carbonate (Na_2CO_3) solution was added to the mixture. The all-prepared solutions were vortexed for homogenization and incubated in the dark for 2 hours. At the end of the incubation process, absorbance measurements were performed at a wavelength of 765 nm using the PG 60 Instrument UV-Vis Spectrophotometer device. The concentrations of the samples were determined using the absorbance values obtained from the gallic acid standard curve (Siddiqui et al., 2017).

2.2.5 Determination of Total Flavonoid Content (TFC)

The Total Flovonoid Content Determination (TFC) method was utilized to determine the total flovonoid contents of the polar microalgae extracts used in this study. For this aim, 20 L of microalgae extract, 20 L of 10% Aluminum Chloride ($AlCl_3$) solution prepared with ethanol solvent, 20 L of 1 M potassium acetate solution prepared with ethanol solvent, and 180 L of distilled water were added to the glass tubes, respectively. Instead of microalgae extract, distilled water was placed in glass tubes as a control with the other solutions remain same amount. The standard solutions created for the calibration curve were supplemented with quercetin standard at the ratios of 0, 10, 25, 50, and 100 μg/mL instead of microalgae extract. Other solvents were added to the glass tubes in the stated amounts, respectively. All prepared samples were incubated for 30 minutes at room temperature. At the end of the incubation, absorbance measurements were taken at 415 nm using the PG 60 Instrument UV-Vis Spectrophotometer. The concentrations of the samples were estimated using the generated quercetin standard curve (Safafar et al., 2015). The chemicals and ratios are summarized in table below.

Table 2.6 Chemicals used in the total flavonoid content analysis

	Blank	**100 μg/mL**	**250 μg/mL**	**300 μg/mL**	**350 μg/mL**	**500 μg/mL**	**1000 μg/mL**
Sample (μL)	-	20	20	20	20	20	20
Distilled water (μL)	20	-	-	-	-	-	-
Ethanol (μL)	20	20	20	20	20	20	20
$AlCl_3$ Solution (μL)	20	20	20	20	20	20	20
Potassium Acetate Solution (μL)	20	20	20	20	20	20	20

2.3 Encapsulation of Polar Microalgae and Characterization

This section involves the electrospraying of flavonoid-rich, polar microalgae extract-loaded nanoparticles as well as the characterisation of the nanoparticles produced. First, an 8% PVA solution in distilled water was prepared for the encapsulation of the polar microalgal extracts collected in the study. To maintain homogeneity, the solution was held in a magnetic stirrer set at 70°C until it entirely dissolved by covering the beaker cap with aluminum foil. Also, a 2% concentration of Alginate (AL) solution was prepared in distilled water. To ensure homogeneity, the solution was held in a magnetic stirrer set at 70°C until entirely dissolved by covering the beaker cap with aluminum foil. Both solutions were transferred to a standard magnetic stirrer without a heating when they reached a completely homogenous consistency. After preparing the solutions, PVA:AL was mixed at a ratio of 80:20 (v/v) and transferred to the magnetic stirrer to achieve a homogeneous consistency. After that, 1% Tween 20 by weight was added to the solution and wait for homogenization, and then 1% polar microalgae extract by weight was added to this prepared solution, respectively. After homogenizing the entire solution, 3 mL of solution is drawn into the injector. Following that, the injector was placed in the electrospraying apparatus in the chamber inside the pump. A metal plate is placed in front of the device and aluminum foil is laid on it. The distance between the plate and the device was fixed to 13 cm. The electrode is then attached to the injector. The device was left to operate after the polymer flow rate was set to 0.25 mL/hr. After the process was finished, the nanoparticles on the aluminum foil placed on the plate were scraped and collected in the epondorf tube. The collected samples were left to stand in an environment that will not receive light and moisture (İnan & Özçimen, 2021; Karakaş et al., 2019).

2.3.1 Field Emission Scanning Electron Microscope (FESEM)

The dimensions of the nanoparticles obtained from the study were measured using a Field Emission Scanning electron microscope (FESEM) in the YTU Central Laboratory at 20-50.00 kX magnification with a Thermo Scientific Apreo 2S LoVAc type FESEM device (Figure 2.12) (İnan & Özçimen, 2021).

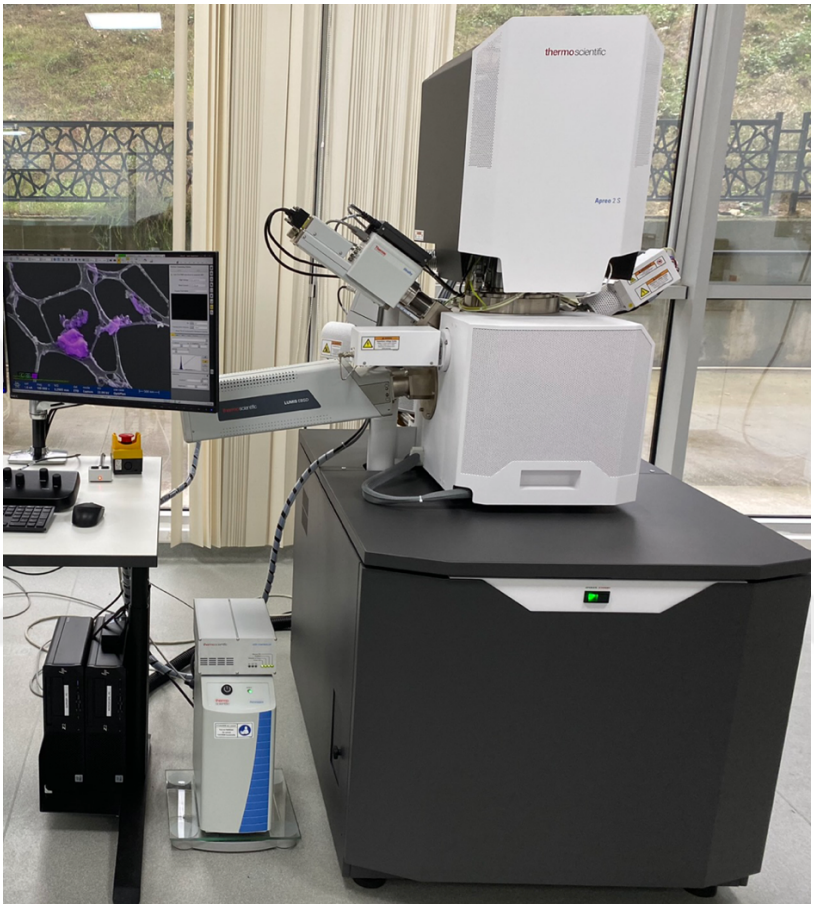


Figure 2.12 Thermo Scientific Apreo 2S LoVAc model FESEM device

2.3.2 Dynamic Light Scattering (DLS)

The particle size and polydispersity index (PDI) of the nanoparticles obtained in the study were determined using the Zetasizer Malvern Zetasizer Nano ZS device. (Figure 2.13) The nanoparticles were gently pulverized before being dissolved in water and passed through a syringe filter with a 0.45 m pore size. Finally, the samples were placed in an ultrasonic-assisted water bath for 10 minutes and their measurements were obtained in the device (İnan & Özçimen, 2021; Tomaszewska et al., 2013).

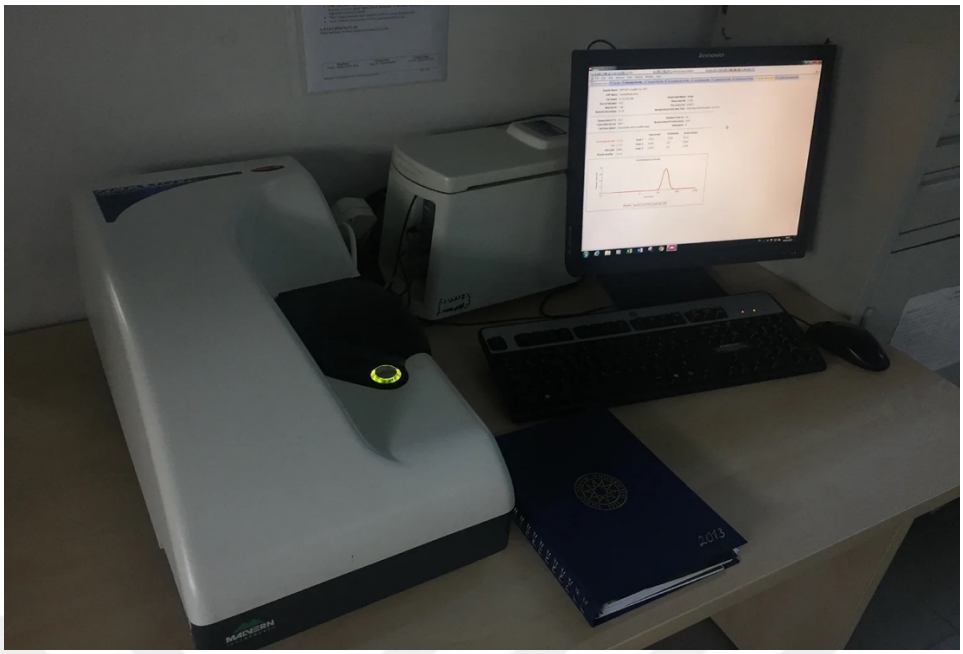


Figure 2.13 Malvern Nano ZS Zetasizer device

2.3.3 Fourier Transform Infrared Spectroscopy (FTIR)

The functional groups of of nanoparticles and determining whether the encapsulation process has taken place were determined using a Bruker brand FTIR device. The ATR technique was used to determine the spectra of the polar microalgae samples utilized in the study at a resolution of of 4 cm^{-1} in the range of 500-4000 cm^{-1} (İnan & Özçimen, 2021; Kiran Kumar et al., 2018).

2.3.4 Thermogravimetric Analysis (TGA)

TG analysis is based on the principle of estimating the weight of materials left after thermal degradation based on temperature variations (H. Khan et al., 2020; Ng et al., 2018). Compounds display stable characteristics in horizontal zones related to temperature on TGA curves. In the experiment, a TA Instrument brand SDT Q600 model thermogravimetric analyzer (Figure 2.14) was utilized to investigate the combustion kinetics of nanoparticles. In the experiment, the device was first turned on and waited for optimized, then around 5 mg of sample was placed in the device, and the weight was expected to be balanced first. Then in a nitrogen atmosphere,

the device was heated to 600°C at a rate of 10°C/min. The curve is generated by changing the sample weight with temperature or time. Throughout the procedure, the nitrogen flow rate was kept constant at 40 mL/min (Koçer & Özçimen, 2021).

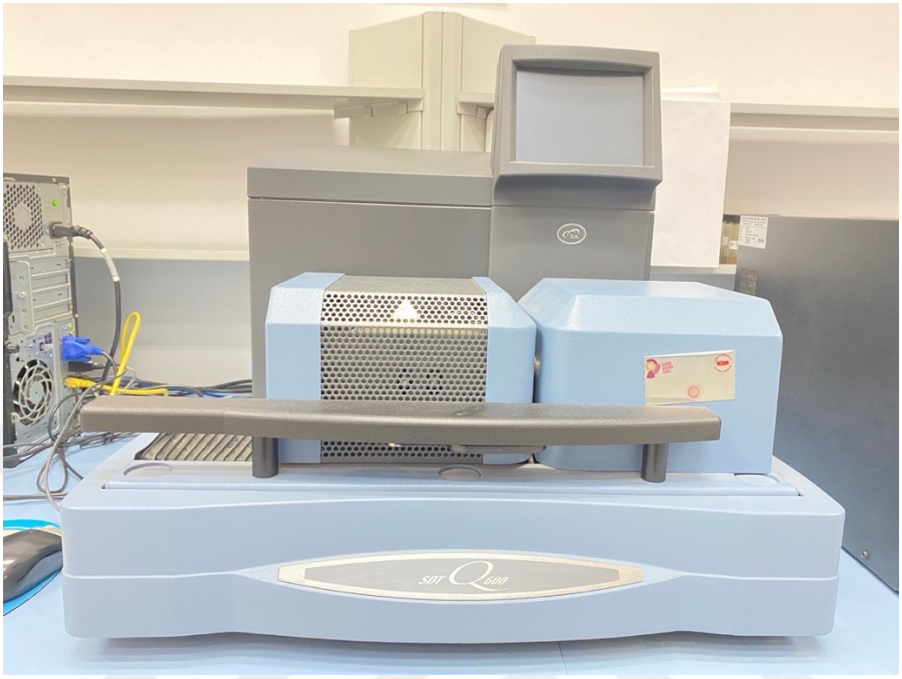


Figure 2.14 TA Instrument brand SDT Q600 model Thermogravimetric analyzer

2.3.5 Encapsulation Efficiency

In order to determine the encapsulation efficiency of the nanoparticles obtained in the study, firstly, 0.5 g/mL stock solution was prepared by dissolving each microalgal extract separately in chloroform. Following that, the stock was diluted, and solutions of 100, 50, 25, and 10 mg/mL were made for the calibration curve. The prepared solutions were placed in a 400°C water bath for 4-5 hours. The UV spectrum was evaluated using the PG 60 Instrument UV-Vis Spectrophotometer device readings and absorbances of the samples were taken against the chloroform blank solution at the determined wavelength. Each nanoparticle produced by the electrospraying procedure was additionally weighed 0.2 mg using a precision scale and dissolved in 4 mL chloroform. Samples with chloroform were kept in a water bath for 4-5 hours set at 40°C. All prepared solutions were centrifuged at 4500 rpm, and the supernatant parts were measured at 270 nm using the PG 60 Instrument UV-

Vis Spectrophotometer. The following equation was used to determine encapsulation efficiency (Karakaş et al., 2019).

$$Encapsulation\ Efficiency\ (EE) = \frac{Total\ amount\ of\ algal\ oil - Free\ amount\ of\ algal\ oil}{Initial\ amount\ of\ algae\ which\ used\ for\ encapsulation}\ x\ 100 \quad (2.11)$$

2.3.6 *In Vitro* Release Experiment

To examine the release profile of the nanoparticles produced during the study, the staining method with Sudan Red G, a water-soluble dye, is used. For this experiment, 8% PVA and 2% AL solutions produced by dissolving in distilled water were utilized for microalgal extract encapsulation. After completely dissolved, PVA and AL were mixed at a ratio of 80:20 (v/v). Tween 20 was then added at 1% by weight to the produced mixture and allowed to mix. Then, instead of 1% microalgal extract by weight, Sudan Red G dye was added to the prepared solution. After that, 3 mL solution was withdrawn to injector and placed in an electrospinning device for the electrospraying process. After electrospraying process, the nanoparticles on the aluminum foil were scraped and collected in ependorf tube. The release medium was prepared with 2% (w/v) Tween 20 dissolved in distilled water. Then, 25 mg nanoparticle was dissolved in 5 mL release medium for use in release profile studies. The resultant mixture was centrifuged at 4200 rpm for 10 minutes using a PG Instruments T60 model refrigerated centrifuge device to determine the initial absorbance measurement. After centrifugation, the absorbance of the supernatant phase was measured at 501 nm using the PG 60 Instrument UV-Vis Spectrophotometer against the release medium as a blank. Then, the mixture was allowed to mix at 150 rpm at room temperature. At specific time intervals (30 minutes, 1 hr, 3 hrs, 7 days, and 10 days), the solution was precipitated using a PG Instruments T60 model refrigerated centrifuge at 4200 rpm for 10 minutes, and absorbance measurements were taken at 501 nm in a PG 60 Instrument UV-Vis Spectrophotometer (Gao et al., 2016).



Figure 2.15 Solution for release profile analysis

2.3.7 X-Ray Diffraction Analysis (XRD)

The crystallographic characteristics and phases of the nanoparticles produced in this study were determined using the PANalytical X'Pert PRO device. The XRD device was used to scan nanoparticles at the room temperature at a scanning rate of 1°C/min in the presence of a copper-K α radiation source (k = 1.54 A˚). During the process, the diffraction angle varied from 10° to 50° 2θ (İnan & Özçimen, 2021).

2.4 Anti-cancer Activity

2.4.1 *In Vitro* Cell Culture

In this thesis study, MCF-7 human breast cancer and A549 human lung cancer cell lines obtained from Adana Alparslan Türkeş University Cell and Tissue Culture laboratory were used. For the study, first of all, cryotube cell lines containing $1x10^6$ cells/mL cells were removed from the cryobank and the thawing procedure was applied. For the thawing procedure, the ISOLAB brand water bath was set to 37°C and the cryotube was placed in the water bath. After the cells in the cryotube were thawed, 5 mL of DMEM (Dulbecco′s Modified Eagle′s Medium) containing 10% (v/v) Fetal Bovine Serum (FBS) and 1% (v/v) penicillin-streptomycin was added to the cells in a Nuaire brand NU-25-400E model sterile cabinet. It was then centrifuged at 2000 rpm for 3 minutes. After centrifugation, the supernatant phase remaining on top was poured and 6 mL of fresh medium was added to suspend the cells remaining in the pellet part. Afterwards, T25 flask cultivation was carried out. Cells in the flask were examined daily under the microscope and grown in a Memmert brand 5% CO_2 incubator set at 37°C until they reached 80-90% density. Cells that reached eighty percent confluence were then passaged.

Passaging was carried out in a sterile cabinet, and firstly the cells were washed with 1.5 mL of sterile Phosphate Buffered Salt (PBS) solution and then treated with Trypsin/EDTA solution. The flask, in which Trypsin/EDTA solution was added to remove the cells from the surface, was kept in a 5% CO_2 incubator set at 37°C, by controlling the removal of the cells from the surface under the microscope at certain times between 5-10 minutes. Then, 3 mL of growth medium is added into the flask and the liquid is taken from the flask with the help of a serological pipette and transferred into the falcon tube. Then, it was precipitated at 2000 rpm for 3 min in a Hettich brand Universal 320R model refrigerated centrifuge set at +4°C. After centrifugation, the supernatant phase was poured and the medium was added to the cell pellet remaining at the bottom, and it was suspended. Before passaging, 1 µl of cells taken from the pellet and 49 µl of trypan blue dye were mixed for cell counting and 100 µl of the mixture was taken into a micropipette. Cells transferred to Thoma slide to counted under Leica DFC295 model Invert microscope. Then, cells were

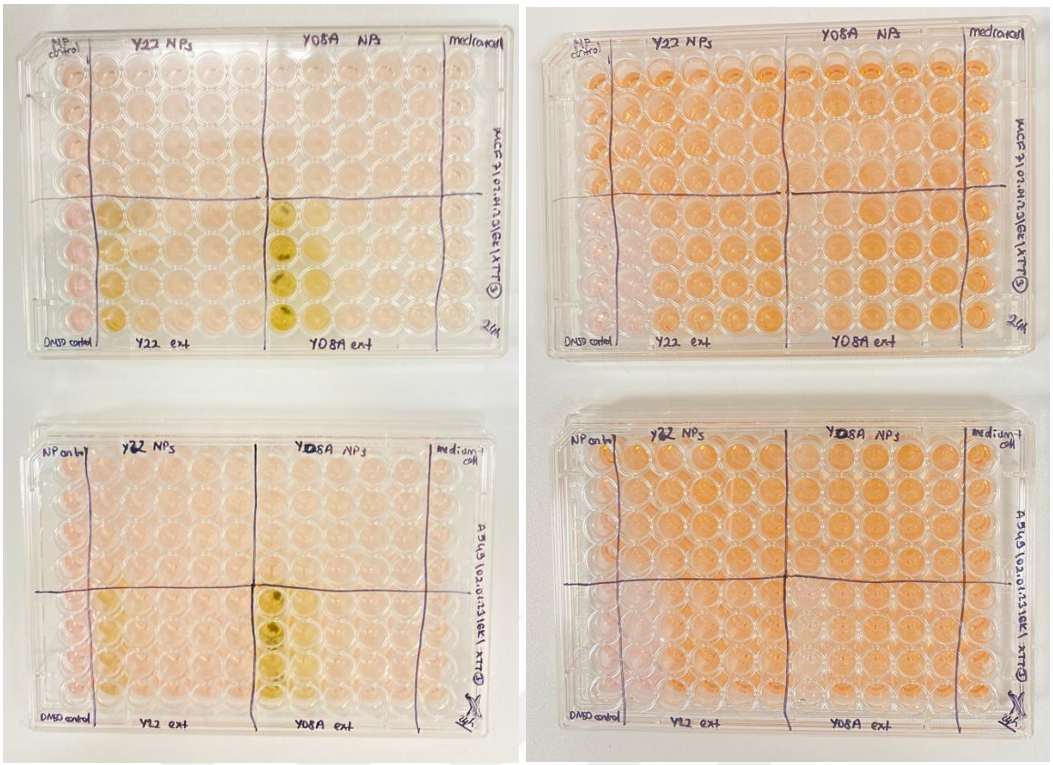
seeded into a T75 flask with 1.5×10^5 cells in each flask, in which the medium was added beforehand, and homogeneous distribution was achieved after cells were added. Passaging cells were inoculated in an incubator with 5% CO_2 set at 37°C (Jia et al., 2014; Rajasekaran et al., 2021).

2.4.2 Cell Viability Analysis (XTT)

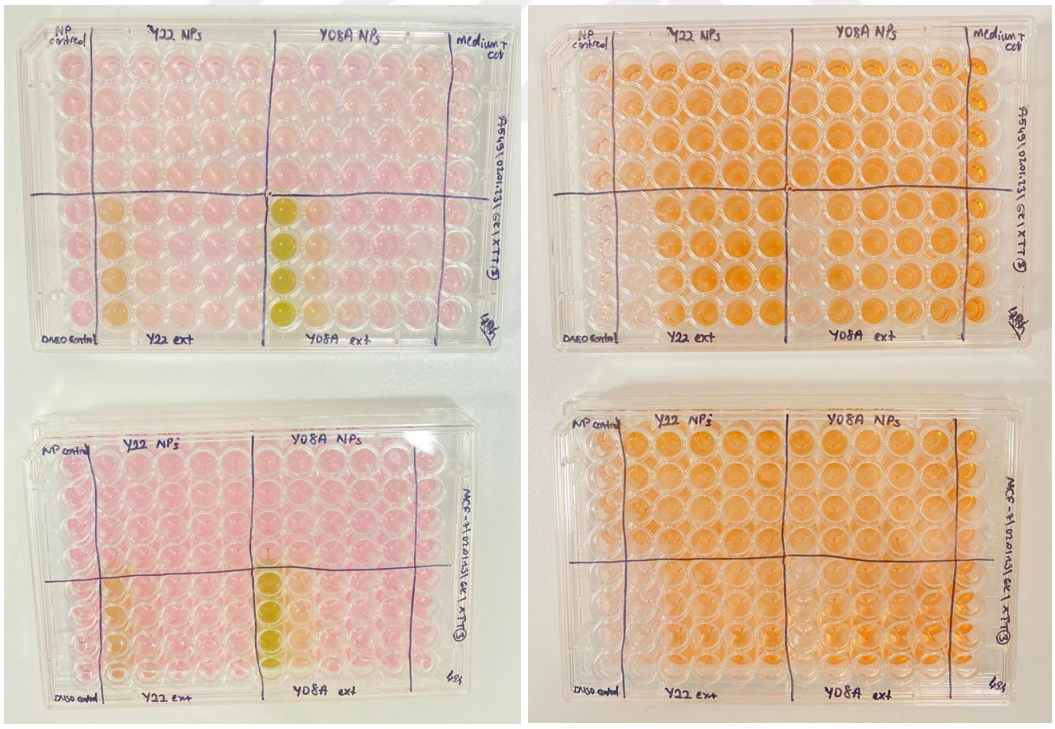
XTT cell cytotoxicity assays [2,3-Bis-(2-Methoxy-4-Nitro-5-Sulfophenyl)-2H-Tetrazolium-5-Carboxanilide] were carried out in accordance with the standards to determine the acticancer activity of polar microalgae extract loaded nanoparticles and polar microalgae extracts prepared at different concentrations. First of all, the determined cell lines were inoculated into a 96-well microplate with $1x10^4$ cells in each well. Afterwards, they were inoculated for 24 hours in an incubator with 5% CO_2 set at 37°C to allow the cells to adhere to the surface until it reached 80-90% confluency. Polar microalgae extracts at different concentrations and nanoparticles loaded with polar microalgae extracts encapsulated by electrospray method were treated on cells. Medium without extract, empty nanoparticle and DMSO were used as controls. Afterwards, the microplate was incubated for 24 hours and 48 hours in separate setups in an incubator with 5% CO_2 set at 37°C. After the incubation, the medium containing the treatment in the wells was withdrawn and washed with PBS. Then, 100 µl of medium and 25 µl of previously prepared activated XTT solution were added to each well. The microplate was kept in an incubator with 5% CO_2 set at 37°C for 2-5 h and the absorbance values were determined by taking measurements in a BMG LABTECH, SPECTROstar® Nano model multi-microplate reader adjusted to 450 nm wavelength (Cree, 2011; İnan, Mutlu, et al., 2023).

Cell viability was calculated with the following formula;

$$Viability\ (\%) = \frac{OD\ of\ treated\ cells}{OD\ of\ control\ cells}\ x\ 100 \qquad (2.12)$$



(A)



(B)

Figure 2.16 XTT plate setups before and after the procedure for 24 h **(A)** and 48 h **(B)**

3
RESULTS AND DISCUSSION

3.1 Cultivation of Polar Microalgae

In this study, firstly, polar microalgae species were grown in 150 mL Erlenmeyer flasks with 10% inoculum under optimal conditions. Daily absorbance measurements were conducted during production, and growth-related data were obtained in accordance with the absorbance values acquired. Growth kinetic calculations were carried out individually for both microalgae species utilizing growth data. Then, the specific growth rates (μ) of the microalgae were calculated using the Monod equation and by using equation 2.1. The doubling time (t_d) value was calculated using the equation 2.2 provided in the method section, with the appropriate growth rate values and displays in Table 3.1. When the growth curves of the microalgae species used in the study are examined, the growth phase stages and adaptation to medium of the microalgae are clearly seen in 15 days.

The growth kinetics curves demonstrate that CP and CS polar microalgae species show a positive increase with adaptation to the environment, and the lag phase of the growth kinetics curves occurs between 0 and 3 days. After adjusting to the environment, microalgae enter the logarithmic growth phase. The growth rate of the microalgae in this phase is influenced by a variety of factors, including temperature, pH, and nutrient concentration. According to the growth kinetics curves, both polar microalgae species grew between 3 and 15 days (Figure 3.2).

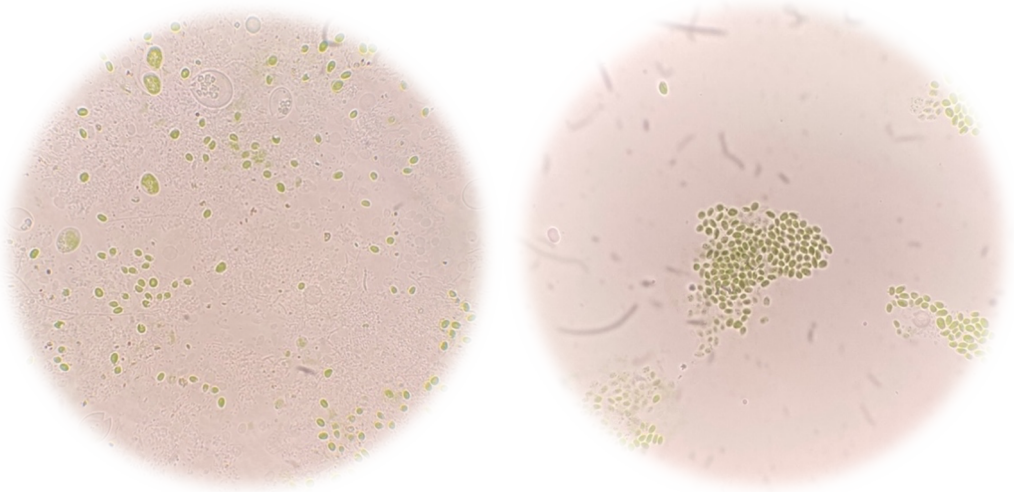
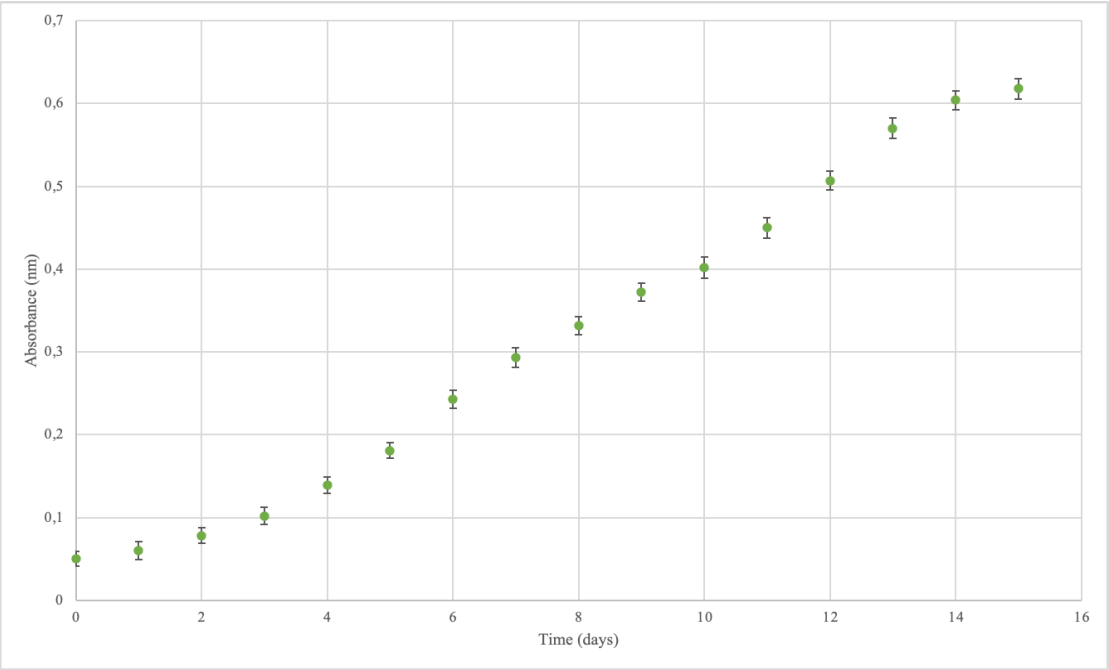


Figure 3.1 CP and CS polar microalgae species under microscope

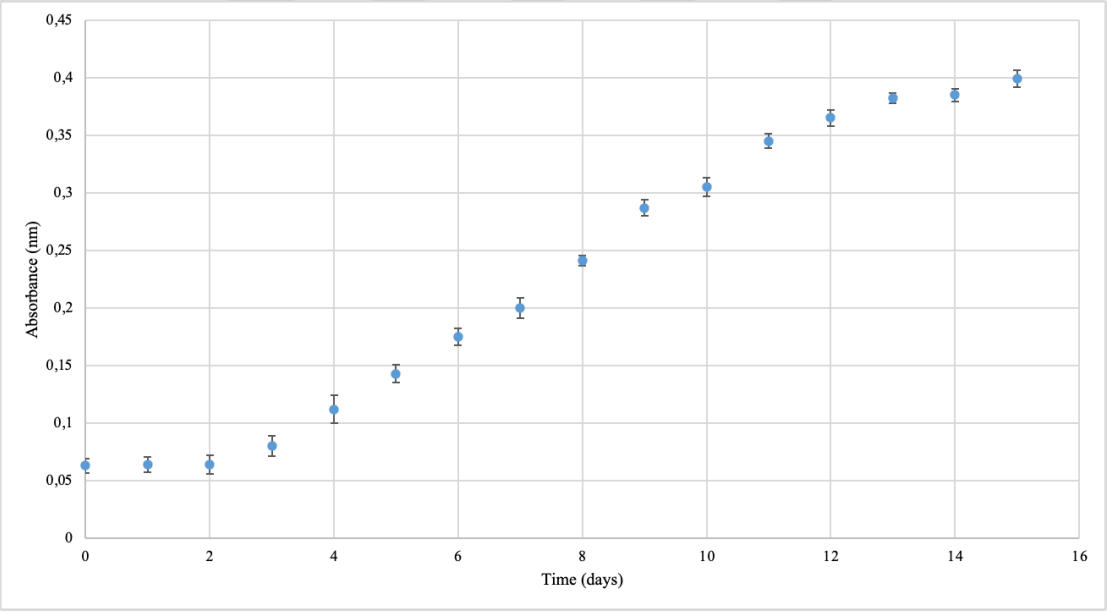
The specific growth rate and doubling time values are calculated based on the information from these curves and are displayed in the table below.

Table 3.1 Growth kinetics of polar microalgae

	Specific Growth Rate	**Doubling Time**
CP	0.16	4.33
CS	0.15	4.62



(A)



(B)

Figure 3.2 Growth curves of CP **(A)** and CS polar microalgae **(B)**

3.1.1 Biochemical Content Analysis

Experiments on the liquid and dry biomass obtained after the cultivation of the polar microalgae species CP and CS were carried out to determine the biochemical content analysis and pigment contents analysis.

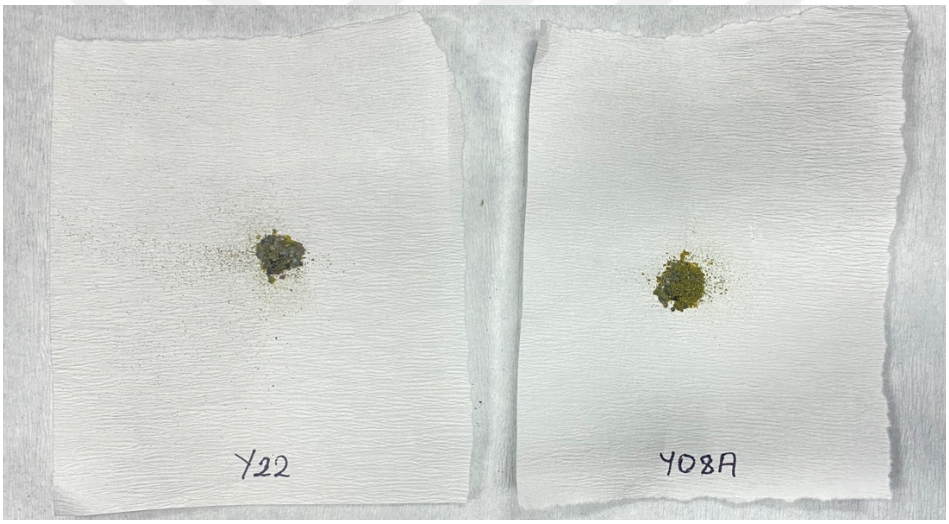
Table 3.2 Results of the chemical content analysis of polar microalgae species

	Carbonhydrate (%)	Protein (%)	Lipid (%)
CP	43.33	30.58	14
CS	35.56	28.06	27

In this context, liquid biomass was then precipitated and dried in a drying oven. The dried solid biomass was milled lightly in a mortar and stored for characterization. Obtained dry biomass was used to conduct carbohydrate and protein content determination experiments (Figure 3.3). As a result of these experiments, carbohydrate content of CP and CS microalgae were found to be 43.33 and 35.56, respectively; and the protein contents were calculated as 30.58 and 28.06, respectively (Table 3.2). In the literature, there are many studies on biochemical content of different microalgae species. Roy and Pal's study compiled a summary of many studies in the literature. They stated that the *Chlorella* sp. microalgae species, which was also used in this thesis, protein content was around 50%, the carbohydrate content was around 15%, and the lipid content was around 14-20% (Roy & Pal, 2015). Molino et al., on the other hand, made comparisons on microalgal species and stated that the protein, carbohydrate and lipid values of *Chlorella vulgaris* microalgae were approximately 45, 5 and 3%, respectively (Molino et al., 2018). Inan et al., used polar microalgae in their study and grown under different conditions. They observed that the carbohydrate content is approximately 20%, the protein content is approximately 30%, and the lipid content is approximately 50% DW under temperate conditions (İnan, 2023). In line with the studies carried out, it is observed that the values obtained in this thesis study are consistent.



(A)



(B)

Figure 3.3 Liquid biomass **(A)** and dry biomass **(B)** samples obtained from CP and CS polar microalgae

Pigments are significant secondary metabolites of microalgae, which generally contain a variety of important properties. According to the studies performed for this thesis, the contents of β-carotene and chlorophyll-*a* were calculated and presented in Table 3.3 For CP and CS polar microalgae, the amount of β-carotene was calculated as 9.11 and 6.14 µg/mL, respectively; and the chlorophyll-*a* contents were calculated as 6.14 and 11.33 µg/mL. In addition, in the study of İnan et al. considering normal conditions, the chlorophyll-*a* content was found to be

approximately 7 µg/mL and the β-carotene content was approximately 2 µg/ml (İnan, 2023). This shows that the values obtained from this study are higher than the literature.

Table 3.3 β-carotene and chlorophyll-*a* contents of polar microalgae

	$\boldsymbol{\beta - carotene}$ **(µg/ml)**	$\boldsymbol{Chlorophyll - \alpha}$ **(µg/ml)**
CP	9.11	19.46
CS	6.14	11.33

3.1.2 Proximate and Elemental Analysis

Thermogravimetric analysis is a quantitative analysis technique that measures changes in the mass of materials that are subjected to temperature in various gas atmospheres over time (Saadatkhah et al., 2020). In line with this method, moisture, ash, volatile matter and fixed carbon contents data, which are also proximate analysis data in the composition of the samples, are obtained (García et al., 2013). In this study, the percentage values of moisture content, volatile matter content, fixed carbon content, and ash content of cultivated polar microalgae were determined as a result of proximate analyses performed using TA Instrument brand SDT Q600 model Thermogravimetric analyzer (Figure 2.14). These results are given in Table 3.4 When the findings of the proximate analysis of the two polar microalgae species are compared, it was found that there are similar outcomes. As a result of combustion kinetic analysis of CP, moisture, volatile matter, fixed carbon and ash contents were determined as 10.23%, 64.33%, 19.71% and 5.73%, respectively. As for the CS, moisture, volatile matter, fixed carbon and ash contents were found as 7.9%, 74.03%, 11.93% and 6.14%, respectively.In accordance with these results, an elemental analysis research was conducted using the following formulas based on dry weight, and the C, H, O, and N values in microalgae content were estimated. These calculated values are displayed in the Table 3.4.

Table 3.4 Proximate and elemental analysis results of CP and CS polar microalgae

	CP	CS
	Proximate Analysis	
Moisture Content (%)	10.23	7.9
Volatile Matter (%)	64.33	74.03
Ash (%)	5.729	6.14
Fixed Carbon Content (%)	19.711	11.93
	Elemental Analysis *	
C	46.2	40.9
H	5.5	5.9
O	36.9	41.4
N	4.9	5.2

***Dry Weight Based**

3.1.3 Fourier Transform Infrared Spectroscopy (FTIR)

Infrared spectroscopy (FTIR/ATR) analysis can identify chemical changes, determine functional groups, and comprehend interactions between nanoparticle surfaces and biologically relevant materials by absorbing IR light and providing a refractive index (Mudunkotuwa et al., 2014; Nourbakhsh & Ashjaran, 2012). In this thesis, the FTIR spectra of CP and CS polar microalgae were obtained using a Bruker brand FTIR instrument, and the absorbance values of the functional groups present in the samples were determined between 500 and 4000 cm^{-1}. Obtained

spectrums are shown in Figure 3.4 and functional groups identified from these spectrums are indicated in Table 3.5.

Table 3.5 Functional groups defined from the FTIR spectrum of polar microalgae

Wavenumber (cm^{-1})	**Fuctional Group**
3730	-OH stretch
3280	-NH stretch
2923	-CH antisym and sym stretching
2856	-CH antisym and sym stretching
1740	C=O stretch
1635	C=O stretch
1539	NO_2 stretch
1453	CH_3 deformation
1383	CH_3 deformation
1240	C – O – C antisym stretch
1152	C – O – C antisym stretch
1033	C – N stretch / P – O – C stretch

As can be observed in Figure 3.4 different functional bands were observed in the FTIR spectrum. Each peak assigned a functional group. The peaks at 3700 cm^{-1} in the spectrum represented the presence of primary amines and very weak secondary amines. Peaks around 3280 cm^{-1} indicates the presence of strong alcohol group. Lipid and carbohydrates appear in the presence of -CH antisym and sym stretching with strong peaks at 2800-2900 cm^{-1}. Strong peaks at 1740 cm^{-1} indicate the lipid peaks of C=O stretching of the carbonyl group in fatty acid esters. Strong peaks at 1635 and 1539 cm^{-1} characterize proteins and amide complexes. The C-O-C stretch

at 1152 cm^{-1} represent the polysaccharides in the microalgae structure. The peaks at 1240 cm^{-1} indicate the presence of nucleic acid. Strong peaks at 1033 cm^{-1} show the presence of carbohydrates and polysaccharides and that nucleic acids carry out phosphodiester bonds with the stretch here (C. Esther Elizabeth Grace et al., 2020; Ponnuswamy et al., 2013; Ss & St, n.d.).

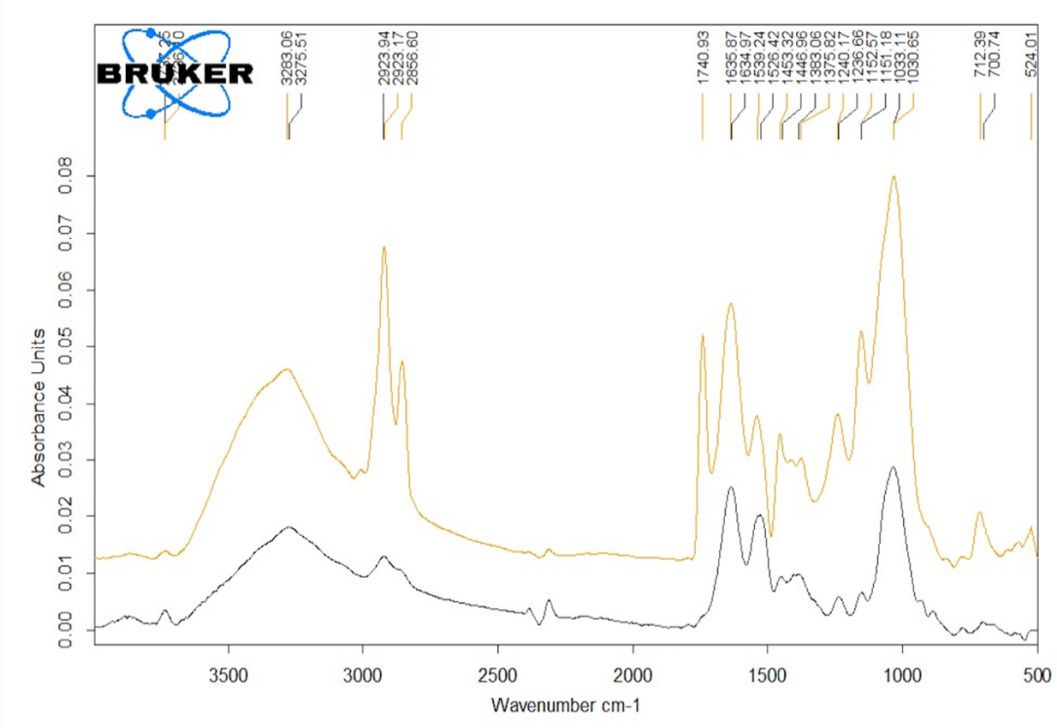


Figure 3.4 FTIR analysis result of CP **(black line)** ve CS **(yellow line)** microalgae

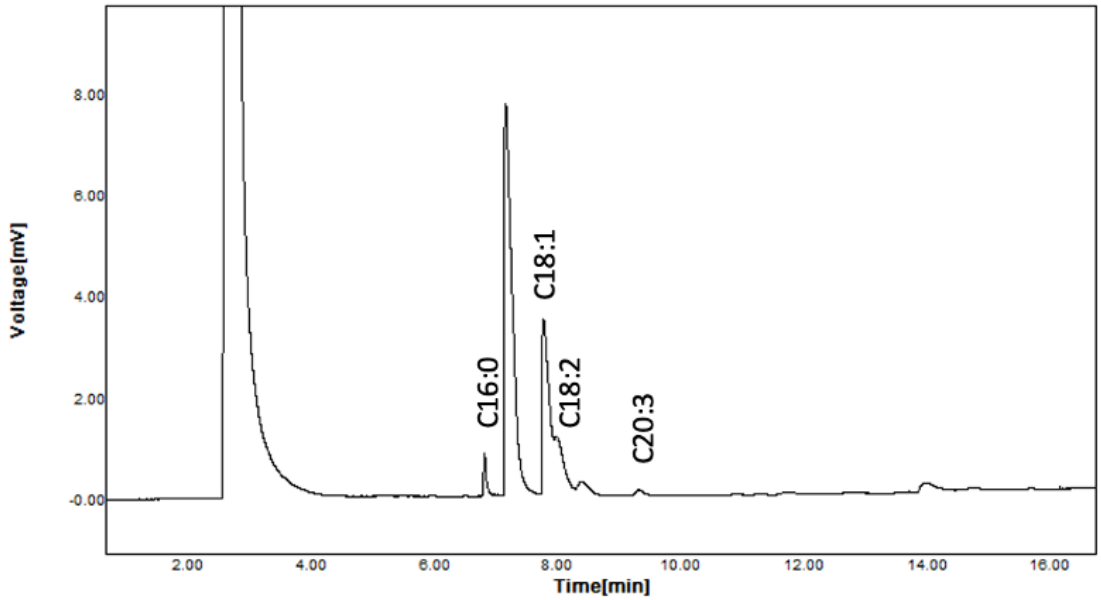
3.2 Extraction of Polar Microalgae

3.2.1 Lipid Content

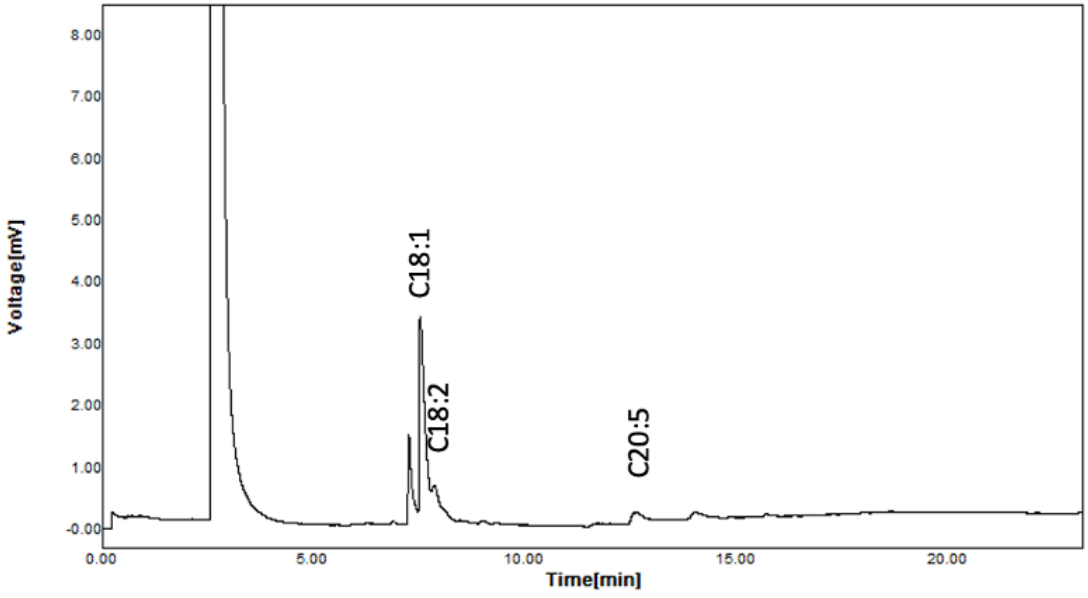
Polar microalgae extracts were obtained from dry biomasses by the Soxhlet extraction method for lipid content analysis. The calculations were based on the weighing findings acquired before and after the extraction process. As indicated in Table 3.2, the percentage of lipid content of CP and CS microalgae was calculated as 14 and 27%, respectively.

3.2.2 Gas Chromatography (GC) Analysis

Gas chromatography is based on the principle of separating compounds in a prepared solution according to their molecular sizes in order to identify and quantify the desired products. As for this thesis study, the fatty acid profile of polar microalgae extracts are determined by GC analysis. When the chromatogram obtained as a result of gas chromatography analyzes of CP and CS polar microalgae oils was examined, it was observed that the fatty acid profile of the samples were similar. According to the obtained chromatogram, the presence of palmitic (16:0), oleic (18:1), linoleic (18:2) and eicosatrienoic acid (20:3) were observed in the polar microalgae oil. Among them, the highest fatty acid content was seen as linoleic acid. The presence of other fatty acids in trace amounts could not be evaluated in the results.



(A)

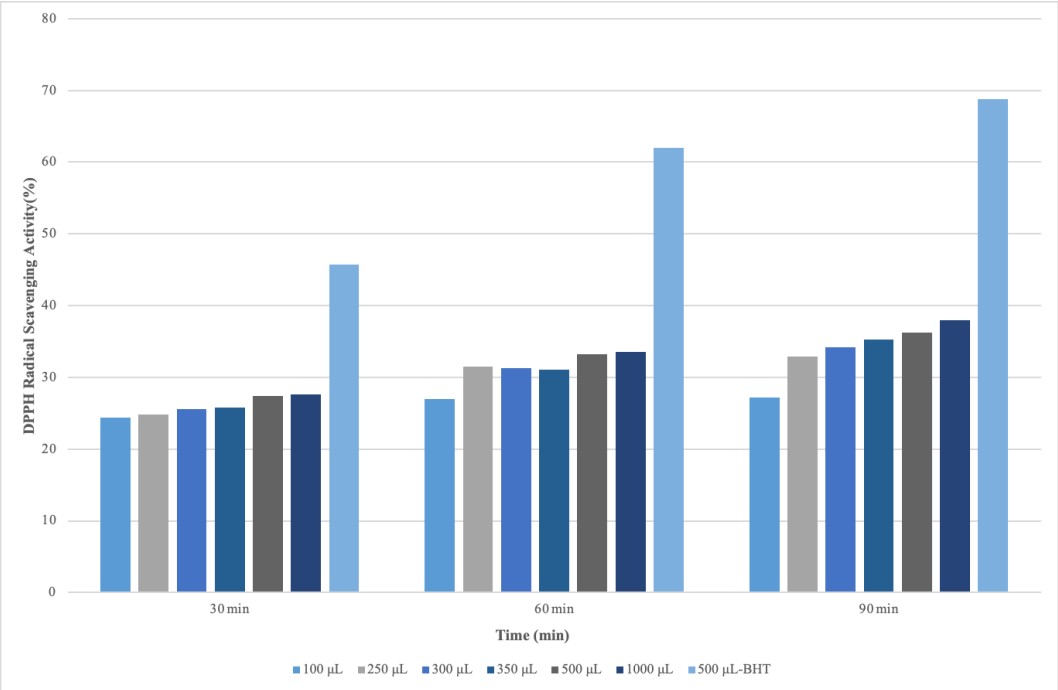


(B)

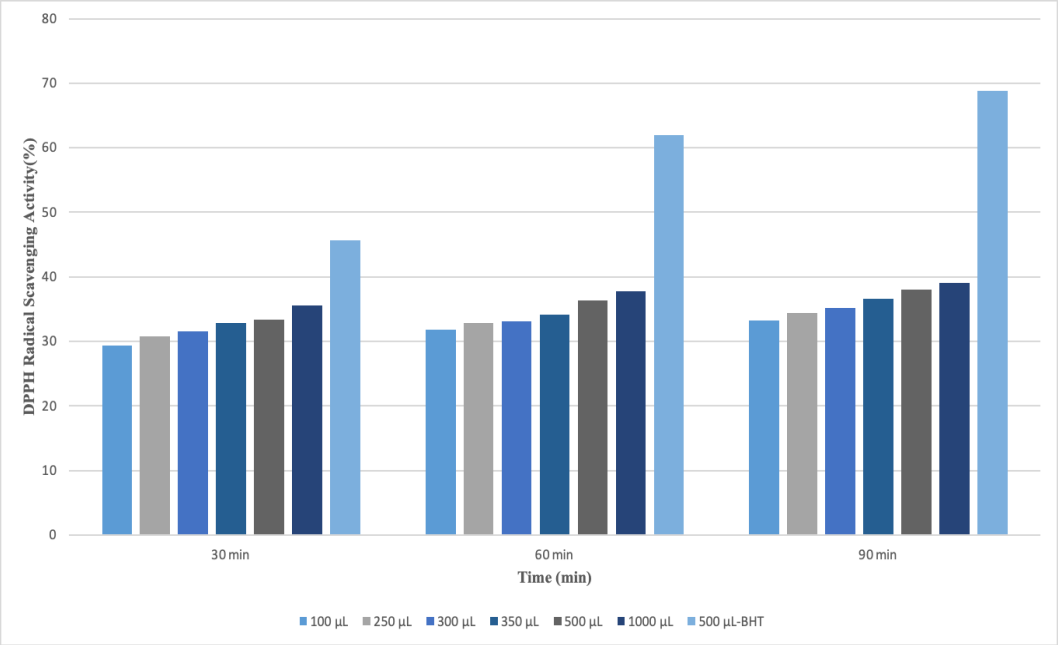
Figure 3.5 Gas chromotography analysis of CP **(A)** and CS **(B)** polar microalgae

3.2.3 Total Antioxidant Activity of Algal Extracts

The DPPH radical is used for evaluating the free radical removal activity of natural antioxidants and provides a calorimetric measurement by changing color in the presence of antioxidants. By using the DPPH free radical removal method, the antioxidant properties of the polar microalgae extract of CP and CS were assessed. The UV-Vis Spectrophotometer was used to measure the solutions made at the predetermined concentrations at 30, 60, and 90 minutes. The results obtained here were displayed on the inhibition-concentration by percentage graph of the radical scavenging activity (Figure 3.6). The DPPH radical, which is normally a dark purple color, starts to turn pink-purple when an antioxidant is added to the medium, at which case it is expected that the spectrophotometer's absorbance would decrease. According to this finding, the antioxidant's ability to scavenge free radicals is higher when the absorbance is lower. In this context, it is acceptable to assume that the antioxidant activity increases as the percent inhibition value rises, in accordance with the values shown on the graph. It was discovered that the microalgae extracts used in this study showed antioxidant capabilities by considering these comments. In addition, the antioxidant activities of the microalgae species used in the study were observed to be similar to each other. In different studies carried out in the literature, it is observed that microalgae have antioxidant properties. Studies on different species give different scavving activity values. When this study is compared with the literature, it is observed that the microalgae species used in this thesis also have antioxidant activity according to the figures below (Ferdous et al., 2023; Senousy et al., 2022).



(A)



(B)

Figure 3.6 DPPH antioxidant activity analysis result of CP **(A)** and CS **(B)** polar microalgae species

3.2.4 Total Phenolic Content (TPC) and Total Flavonoid Content (TFC) Determination

The main purpose of the study is to investigate the anticancer properties of polar microalgae. Due to their significant antioxidant, anti-inflammatory, and bioactive properties, flavonoids—natural pigments found in the rich contents of polar microalgae—can be used against cancer cells in this context (Ferdous & Balia Yusof, 2021; Kopustinskiene et al., 2020; Pandey & Khan, 2021). Flavonoids are also referred to as natural phenolic compounds (Mutha et al., 2021). The total phenolic contents of CP and CS polar microalgae extracts were quantified using the TPC method in accordance with this study. The concentration was calculated using this method using the gallic acid standard curve (Siddiqui et al., 2017), and the results are displayed in Table 3.6.

Table 3.6 Phenolic and flavonoid contents of CP and CS polar microalgae

	Phenolic Content **(GAE/g DW)**	***Flavonoid Content*** **(Q/g DE)**
CP	43.19	1.16
CS	42.04	0.6

In conclusion, the phenolic contents of CP and CS polar microalgae extracts were calculated as 43.19 mg GAE/g DW and 42.04 mg GAE/g DW, respectively. Haoujar et al. al., investigated the total phenolic values on four different microalgae species, and the TPC result was approximately 30-40 mg/g GAE (Haoujar et al., 2019). In another study Hajimahmoodi et al., was performed TPC analysis on different microalgae species in different solvents. Although the results are generally low, the highest total phonolic value was obtained from *C. vulgaris* as 19.15 ± 0.04 mg GAE g^{-1} (Hajimahmoodi et al., 2010). Andriopoulos et al., examined the total phenolic values of many microalgae species and the highest result was obtained as 9.04 ± 0.68 mg GAE g^{-1} DW for *C. minutissima* at the early stationary phase

(Andriopoulos et al., 2022). In the study from Safafar et al., the TPC values obtained from different species were found in the range of 7.72 ± 0.08 to 3.16 ± 0.04 mg/g GAE (Safafar et al., 2015). The phenolic contents of the polar microalgae species used in the study was found to be high when compared to values in the literature. So that, flavonoids in phenolic contents can also be found in high amounts.

The flavonoid contents of CP and CS polar microalgae extracts were quantitatively determined by TFC method. In order to apply this method, firstly, a calibration curve (Figure 3.7) was obtained from the quercetin standard, which is a flavonoid compound. The flavonoid contents of CP and CS polar microalgae extracts were calculated as 1.16 mg Q/g DE and 0.6 mg Q/g DE, respectively (Table 3.6).

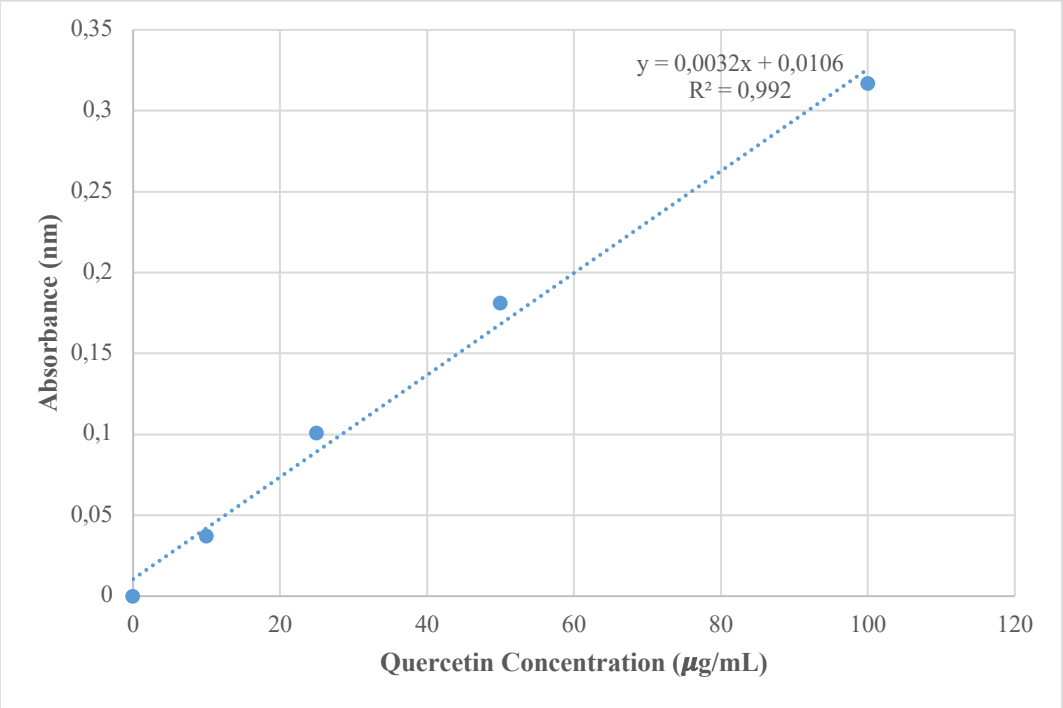


Figure 3.7 Quercetin standard calibration curve

In the study carried out by Grubišić et al., using five different microalgae species, the highest TPC and TFC values are obtained from *Nitzschia sp.* 22.64 ± 1.86 mg GAE/g DW and 0.67 ± 0.10 mg QE/g DW, respectively (Grubišić et al., 2022).

In the study carried out on different species, the highest TFC value was found as 5.18 ± 0.07 mg/g QE (Haoujar et al., 2019); and in another study, it was obtained

as 4.03 ± 1.10 mg/g QE (Safafar et al., 2015). The flavonoid content of the polar microalgae species used in this study was found to be relatively high when compared to the data in the literature. The Antarctic microalgae species was discovered to have a greater flavonoid content than the other polar species.

3.3 Encapsulation and Characterization of Polar Microalgae

In accordance with this study, polar microalgae extracts were encapsulated primarily to prevent lipid oxidation, but also to optimize the dose regimen, reduce systemic toxicity, and specificity to tumor cells (Bonferoni et al., 2017). In this context, polar microalgae extracts were electrosprayed with an 80:20 (v/v) PVA:AL polymeric solution (Figure 3.8), and characterisation studies of the resulting nanoparticles were carried out. These investigations include Field Emission Scanning Electron Microscopy (FESEM), Dynamic Light Scattering (DLS), Fourier Transform Infrared Spectroscopy (FTIR), Thermogravimetric Analysis (TGA), X-Ray Diffraction Analysis (XRD), encapsulation efficiency, and *in vitro* release experiments.

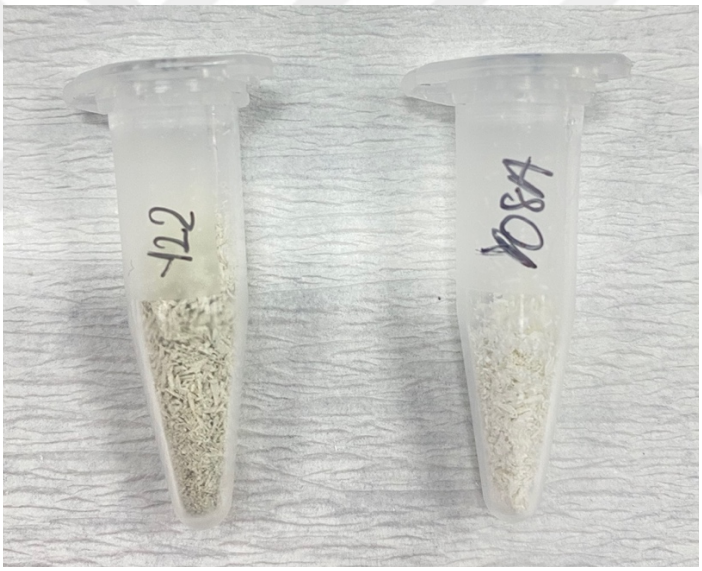
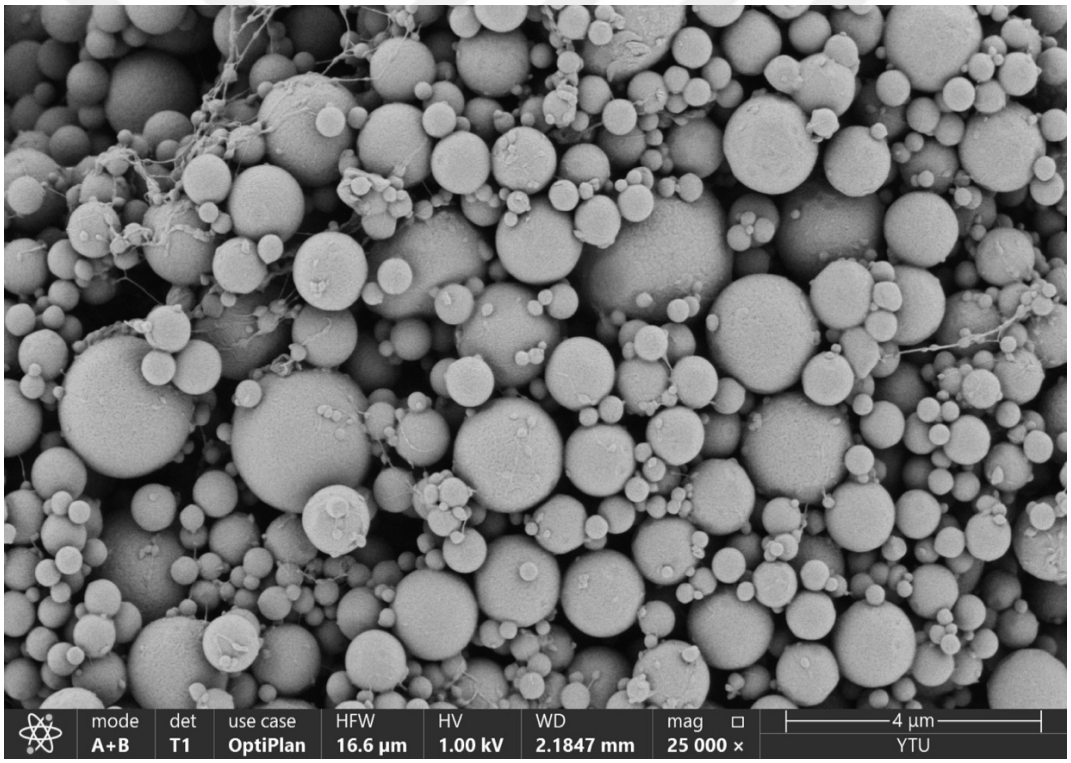


Figure 3.8 Nanoparticle samples obtained by encapsulation with electrospraying

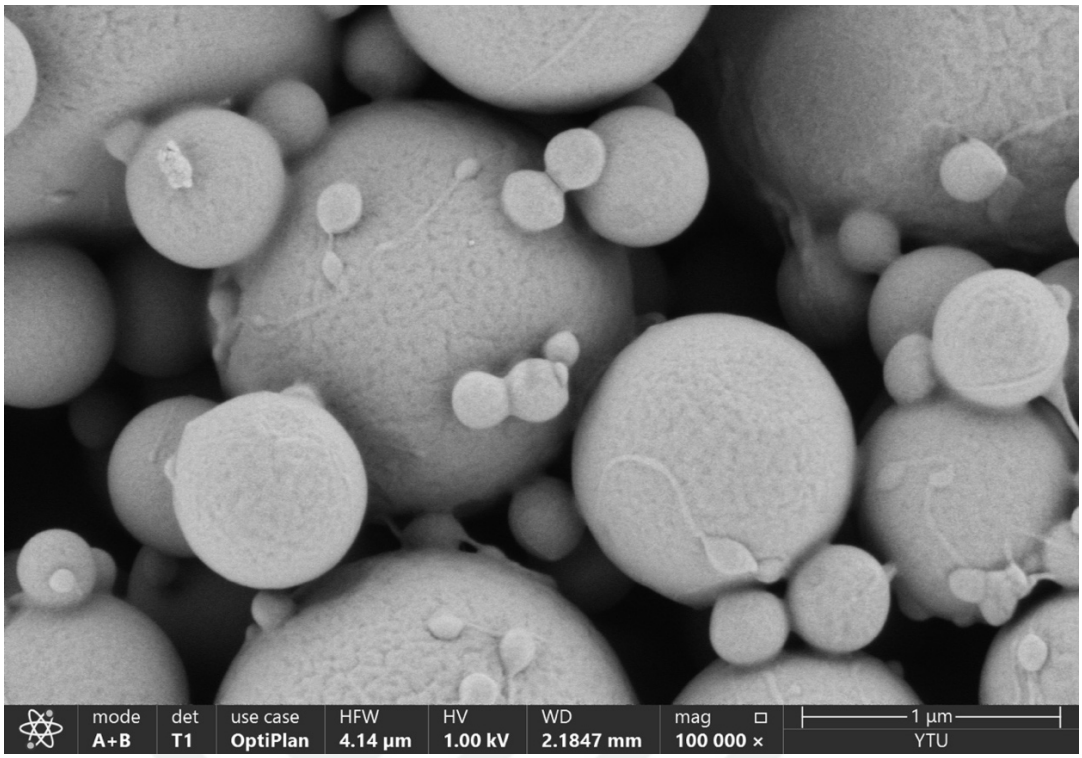
3.3.1 Field Emission Scanning Electron Microscope (FESEM)

The size and shape of the polar microalgae extract-loaded nanoparticles that were obtained for this thesis were determined using the field emission scanning electron microscopy (FESEM) analysis method. Thermo Scientific Apreo 2S LoVAc model FESEM device (Figure 2.12) was used in the Central Laboratory of Yıldız Technical University to analyze nano-size structures using a 1.5 nm thin Au-PD coating. FESEM examination revealed that the obtained particles were spherical. Considering the FESEM results, it is observed that very high resolution and spherical nanoparticles are obtained (Figure 3.9) (Chaturbedy et al., 2015; Pudukudy & Yaakob, 2015).

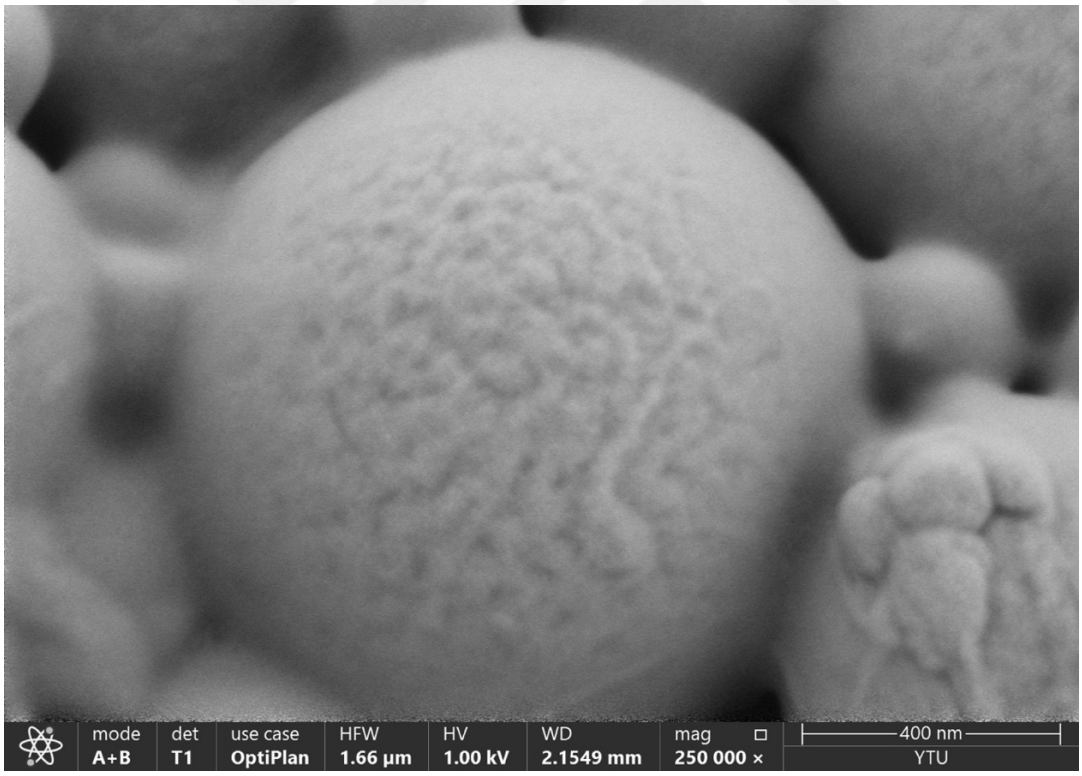


(A)

Figure 3.9 FESEM analysis pictures of polar microalgae extracts-loaded nanoparticles at 25 kX magnification **(A)**, 100 kX magnification **(B)** and 250 kX magnification **(C)**



(B)



(C)

Figure 3.9 FESEM analysis pictures of polar microalgae extracts-loaded nanoparticles at 25 kX magnification **(A)**, 100 kX magnification **(B)** and 250 kX magnification **(C)** (continued)

3.3.2 Dynamic Light Scattering (DLS)

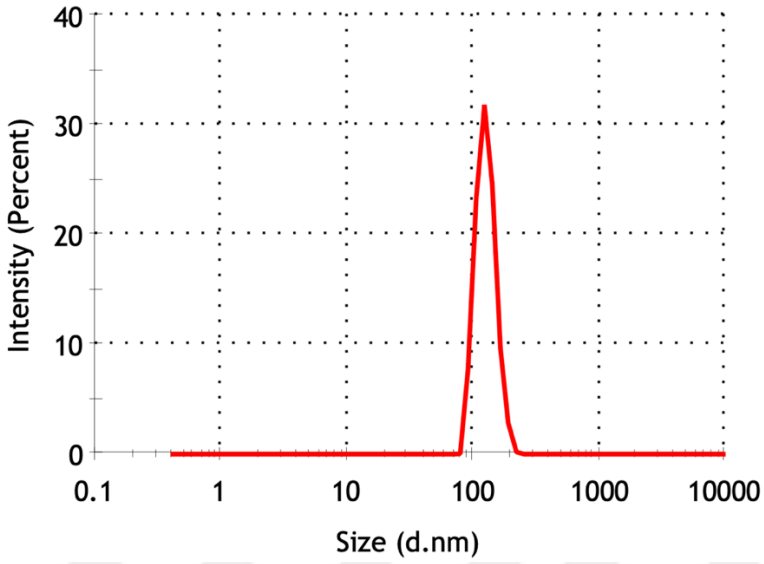
The Dynamic Light Scattering technique is commonly used to assess the particle size of nanoparticles. Light is scattered from particles suspended in a liquid solvent due to random Brownian movements. The size of the nanoparticles is determined by detecting the change in scattered light with the Zetasizer device (Hoo et al., 2008). The size distribution of polar microalgae extracts-loaded nanoparticles obtained under optimal conditions and filtered before being processed by DLS technique using Malvern Nano ZS Zetasizer device (Figure 2.13).

Table 3.7 Nanoparticle size analysis results after DLS method

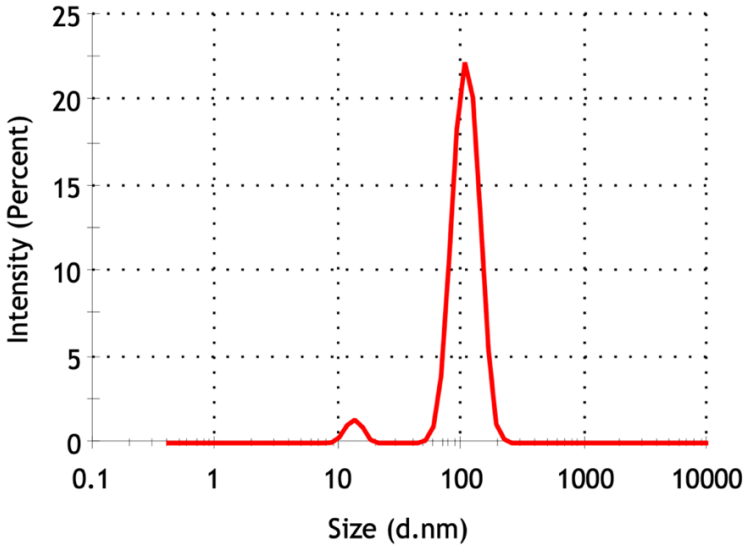
	$\boldsymbol{Z-ave\ (d.nm)}$	$\boldsymbol{PdI}$
CP	294.7	0.406
CS	156.4	0.401

In line with the results obtained, CP loaded nanoparticles and CS loaded nanoparticles sizes and polydispersity index (PDI) values given in Table 3.7. When the DLS curve is examined (Figure 3.10), it is seen that these results peak at a single point. While the average diameter of the nanoparticles loaded with CP was calculated as 294.7 nm and the PdI value as 0.406; the average diameter of the nanoparticles loaded with CS microalgae extract was calculated as 156.4 nm and 0.401 in PdI value. These findings demonstrated that the generated nanoparticles were of sufficient size to be considered as nanoparticles and can be used in many applications (I. Khan et al., 2019; Prabha et al., 2016; Ray et al., 2009). The low PDI value also shows that the NPs' size distributions are more similar to one another (Ribeiro et al., 2020). In another study, the average particle size of the microalgae coated with PVA/AL was calculated under different conditions. Average particle size of *M. aeruginosa* microalgae was found between 90-308 nm while the average particle size *B. braunii* microalgae found as between 197-463 nm, both with low

PdI values. Also, it was observed that the increase in PVA concentration decreased the particle size (İnan & Özçimen, 2021). The results of the FESEM analysis are also supported by the outcomes of the dimensional measurement analysis of Zeta-sizer.



(A)



(B)

Figure 3.10 Size distribution by intensity results by DLS analysis of CP loaded nanoparticles **(A)** ve CS loaded nanoparticles **(B)**

3.3.3 Fourier Transform Infrared Spectroscopy (FTIR)

The absorbance values of the functional groups present in the samples and the FTIR spectrum of CP and CS polar microalgae loaded nanoparticles were determined in the 500-4000 cm^{-1} range. The obtained spectrums are shown in Figure 3.11, and the functional groups identified from these spectrums are indicated in Table 3.8.

Table 3.8 Functional groups defined from the FTIR spectrum of polar microalgae loaded NPs

Wavenumber (cm^{-1})	**Fuctional Group**
3300	-NH stretch
2930	-CH antisym and sym stretching
1723	C=O stretch
1611	C=O stretch
1420	C – N stretch
1334	NO_2 sym stretch
1088	C – N stretch
933	CH_2 out-of-plane wag
841	CH out-of-plane wag

As can be observed in Figure 3.11, different functional bands are observed in the FTIR spectrum, and each represents a different functional group. In these bands, lipid, carbohydrate, protein groups, which were also observed in previous results with polar microalgae extracts, are observed and these bands overlap (C. Esther Elizabeth Grace et al., 2020; Ponnuswamy et al., 2013; Vidovic et al., 2022).

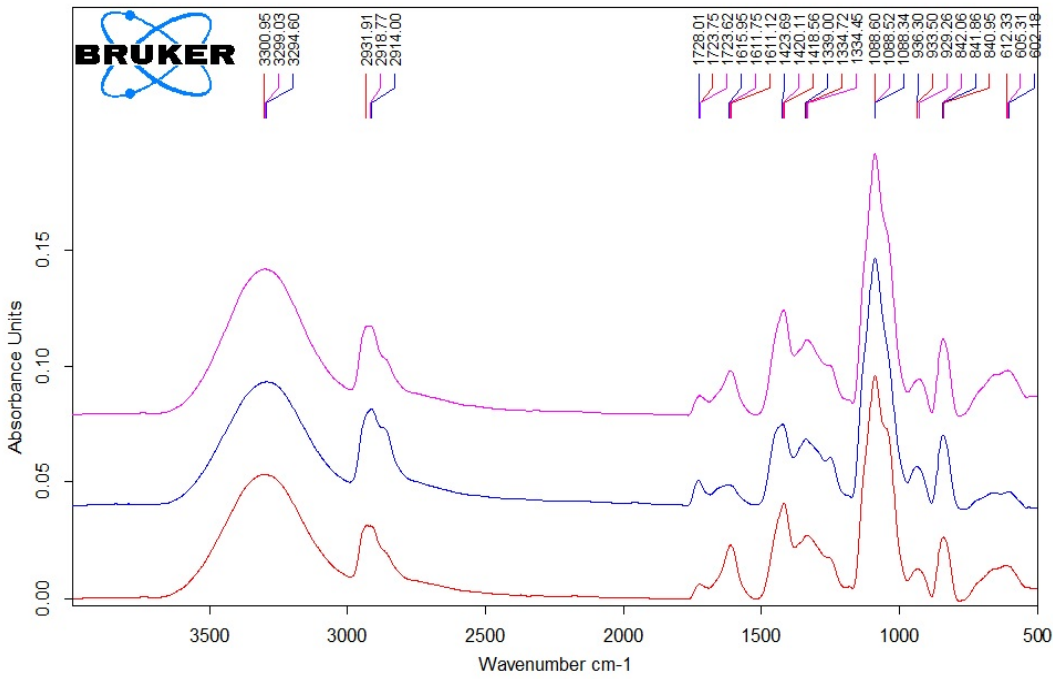
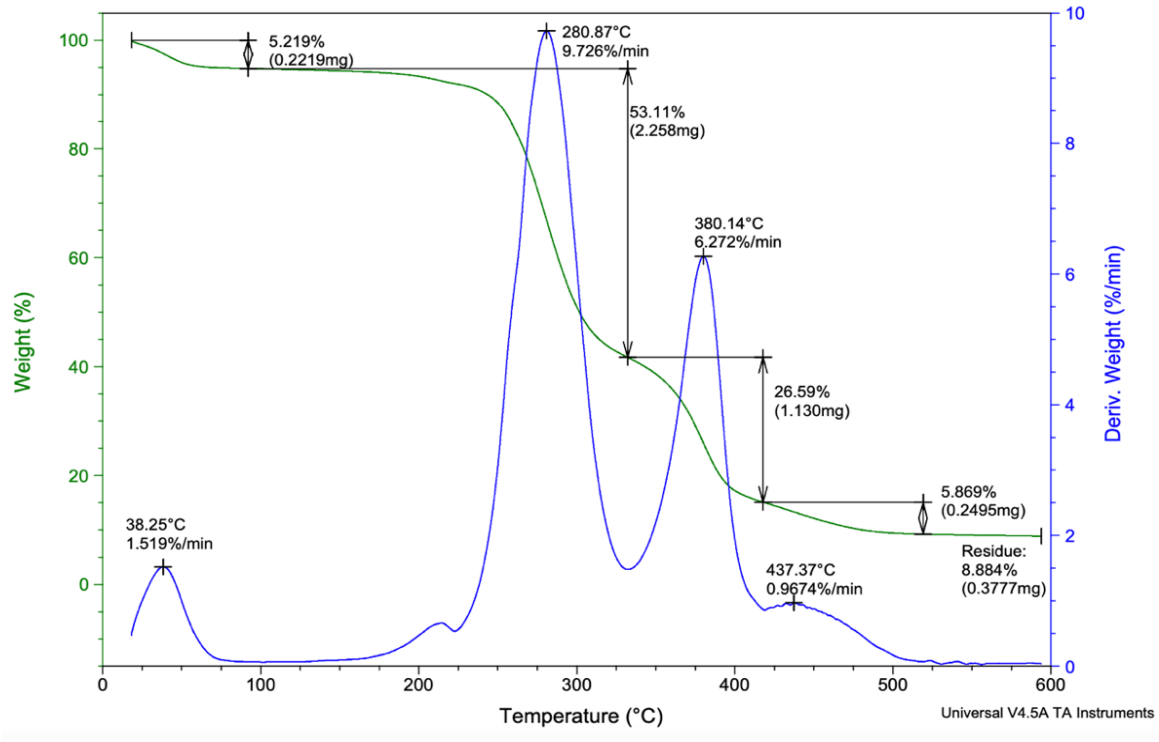


Figure 3.11 FTIR analysis result of CP loaded nanoparticles **(pink line)** ve CS loaded nanoparticles **(red line)** and null nanoparticles **(blue line)**

This overlap indicates the presence of oil in the content of the produced nanoparticles. Therefore, it can be said that polar microalgae extract is encapsulated in this direction. When the literature was examined, it was found that İnan and Özçimen encapsulated microalgae extracts with PVA-AL (İnan & Özçimen, 2021), NiO-NP was obtained from microalgae extract in the study of Moavi et al. (Moavi et al., 2021) and N. Abdel-Raouf et al. obtained Ag-NP from microalgae powder in line with the FTIR spectrum (Abdel-Raouf et al., 2018). Also, the peaks obtained at 3300, 2930 and 1420 cm^{-1} are typical FTIR spectra of the PVA coating; The peak obtained at 1334 cm^{-1} indicates the presence of AL. The peak observed at 1090 cm^{-1} is a result of PVA-AL combination and shows us that our coating material is obtained properly. (Duru Kamaci & Peksel, 2021; Yumin et al., 2022).

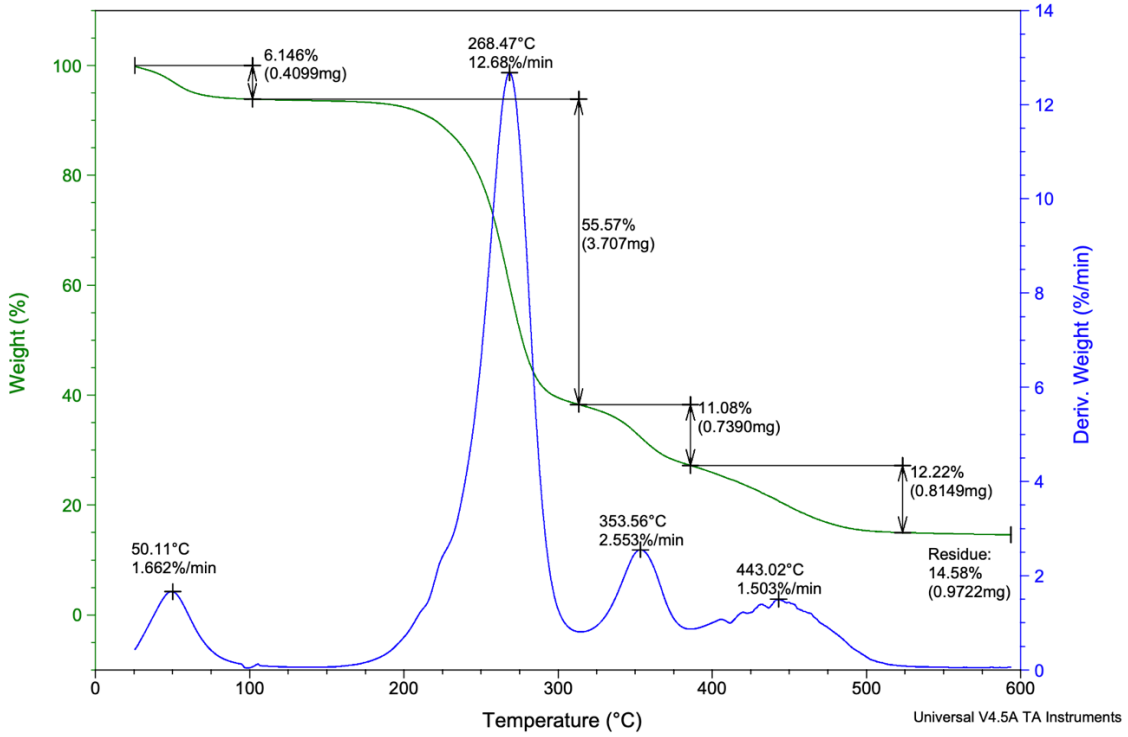
3.3.4 Thermogravimetric Analysis (TGA)

Thermogravimetric analysis shows the physical or chemical properties of the sample over time while it is exposed to a temperature in various gas atmospheres. During this analysis, the sample mass, duration, and temperature are monitored and recorded promptly via the monitor, and as a resulting ploting a graph (Figure 3.12) (Saadatkhah et al., 2020). Weight variations in polymeric materials during TGA analysis can be caused by degradation and oxidation reactions in their structure, as well as physical processes (Ng et al., 2018). Additionally, reactions allow us to understand the composition, degradation mechanisms, reaction kinetics, and organic-inorganic properties of materials that exhibits mass loss or gain (Nasrollahzadeh et al., 2019, 2019). This study provided information on the structural changes of nanoparticles coated with polymeric material as a result of thermal degradation. As a result, the degradation of polar microalgae extract-loaded nanoparticles used as possible biotechnological drugs in the future can be estimated (Loganathan et al., 2017).

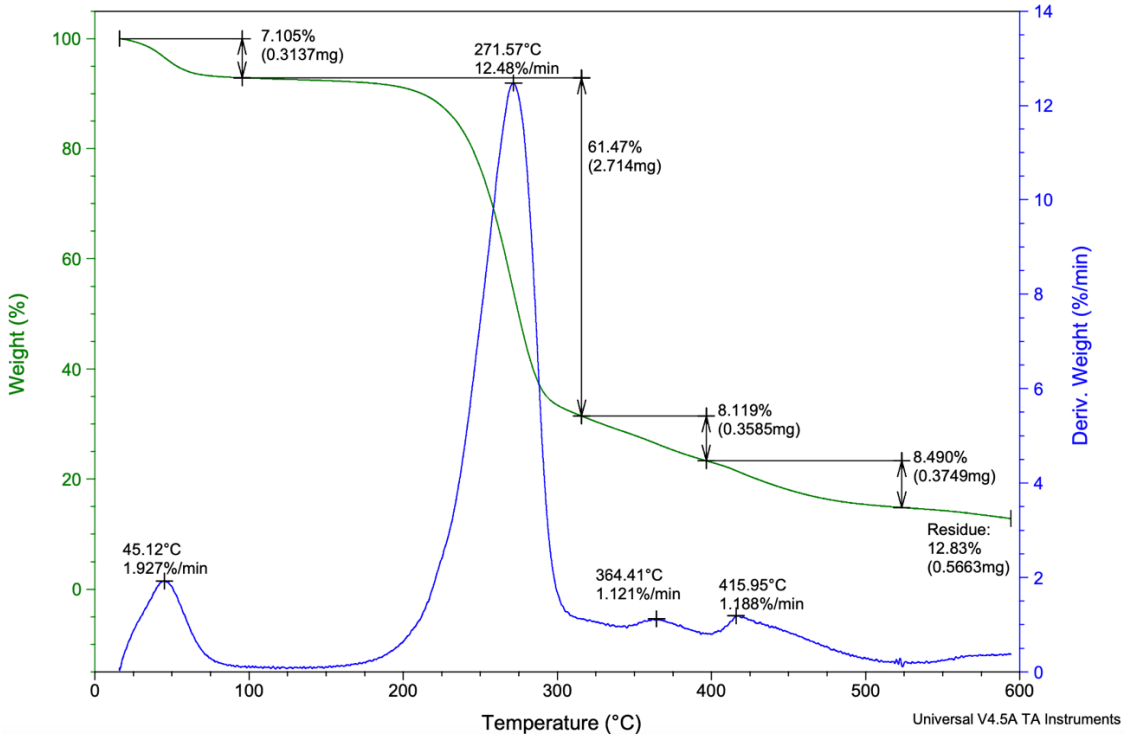


(A)

Figure 3.12 Thermogravimetric analysis result of null-NPs **(A),** CP loaded NPs **(B)** and CS loaded NPs **(C)**



(B)



(C)

Figure 3.12 Thermogravimetric analysis result of null-NPs **(A),** CP loaded NPs **(B)** and CS loaded NPs **(C)** (continued)

Both resulting microalgal extract-loaded nanoparticles began to degrade slowly at a temperature of about 225°C. In microalgae oil-free empty nanoparticles, residue was determined to be 8.9%; while nanoparticles loaded with polar microalgae extract had a residue of 12–14%. The degradation of the nanoparticles occurs in two stages, as can be seen when the TGA curve is examined after the analysis. First, at the temperature range of 225 to 275°C, the nanoparticles showed a weight loss of 61% and 55%, respectively, while the empty nanoparticle showed a weight loss of 53%. In the second step, degradation takes place in the temperature range of 275 to 600°C. An 18-23% weight loss occurred in NPs loaded with microalgae extract, whereas a 32% weight loss occurred in empty NPs. The first diffraction is assumed to have resulted from the thermal degradation of PVA, whereas the second diffraction is thought to have resulted from the by-product formation of PVA. Furthermore, the TGA curve reveals that empty nanoparticles degrade faster and lose more weight than microalgae extract-loaded nanoparticles.

3.3.5 Encapsulation Efficiency and *In Vitro* Release Experiment

The encapsulation efficiency experiment of the nanoparticles in accordance with the study was carried out. It was evaluated whether our microalgae extracts were loaded into the PVA:AL polymeric matrix and how efficiently they were loaded. The encapsulation efficiencies of nanoparticles loaded with CP and CS extracts produced as a result of the thesis study were determined to be 85% and 92%, respectively. If the obtained results are compared with the literature, it is observed that very high encapsulation efficiency is obtained (Blanco-Llamero et al., 2022; İnan & Özçimen, 2021). This demonstrates that the encapsulation procedure has been carried out effectively and provides data to validate the findings of other analysis results. *In vitro* release experiment was carried out to obtain information on the release mechanism of the substance in the nanoparticles produced. In this manner, preliminary information on the release kinetics in the body of obtained nanoparticles will be understood and they will be used as potential biotechnological treatment for cancer disease in the future. In this context, *in vitro* release experiments were carried out using Sudan Red G dye and the obtained results were shown in Figure 3.13.

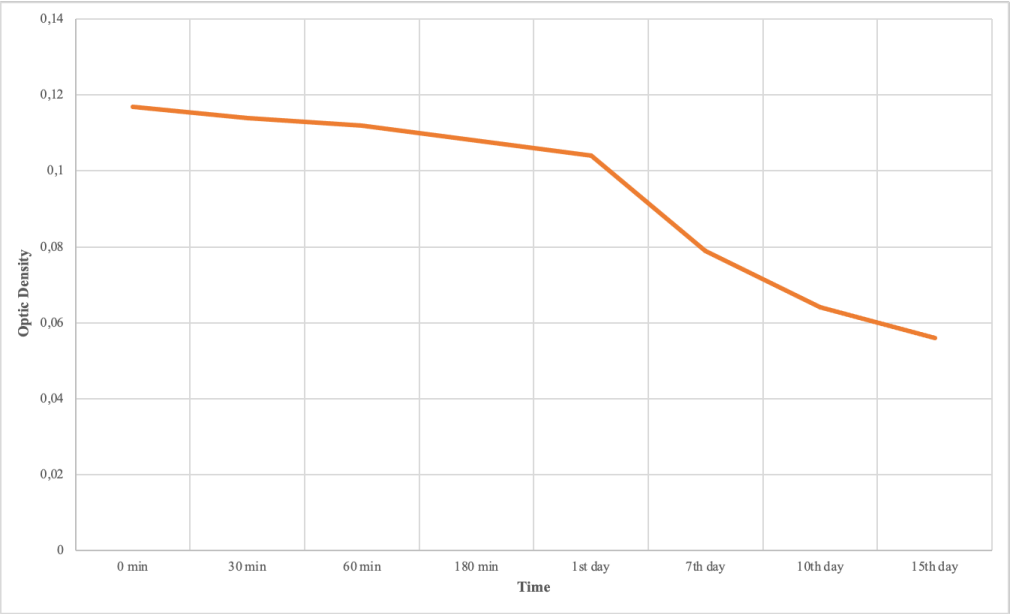


Figure 3.13 Release profile analsis results using Sudan Red G dye

Significant amount of dye held in the capsule was released in a controlled manner after 24 hours, as seen in the figure above. This indicates that the encapsulated bioactive will be effectively released within the first 24 hours. Also, this oscillation happened gradually, rather than abruptly. In this approach, it has been seen that the nanoparticle obtained in the study will be released in a controlled manner in the body when utilized as a potential biotechnological drug in future investigations.

3.3.6 X-Ray Diffraction Analysis (XRD)

The pysico-chemical properties of the crystallite structures used in encapsulation and their size have been identified according to the peaks of the X-Ray diffraction analysis. In this approach, analyses of nanoparticle size, such as FESEM and DLS, are supported. As can be seen in Figure 3.14, the Bragg Angle 2θ peak is observed between 19-20° (H. Khan et al., 2020; Modena et al., 2019). The peaks observed in the figure indicate the amorphous structure **(Biswas et al., 2018; İnan, Koçer, et al., 2023)**.

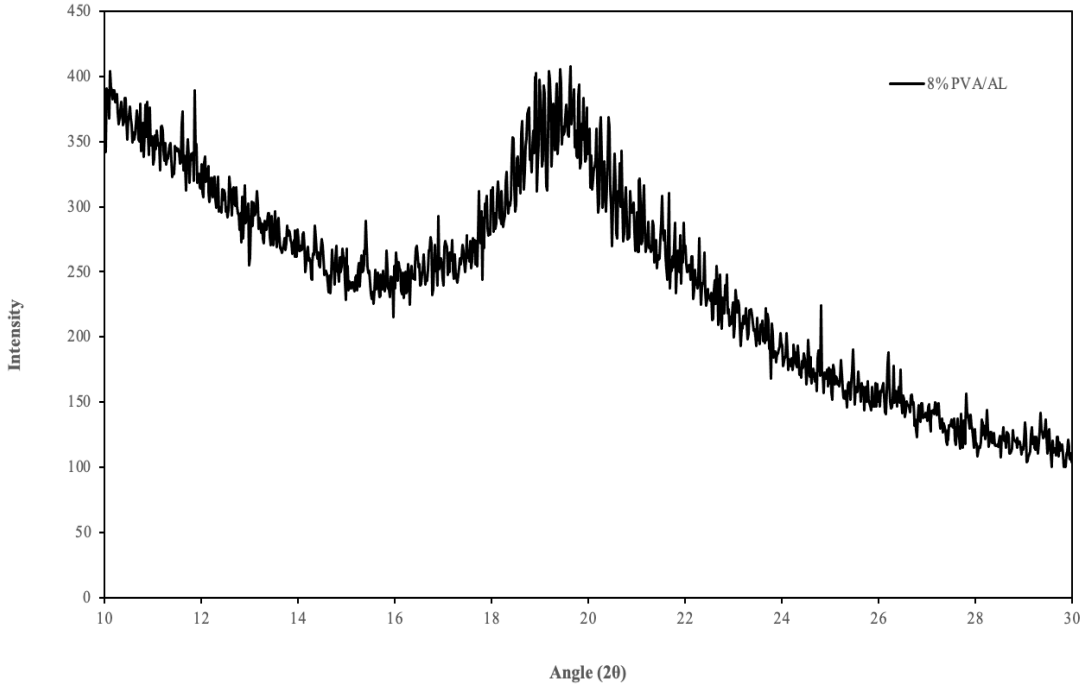
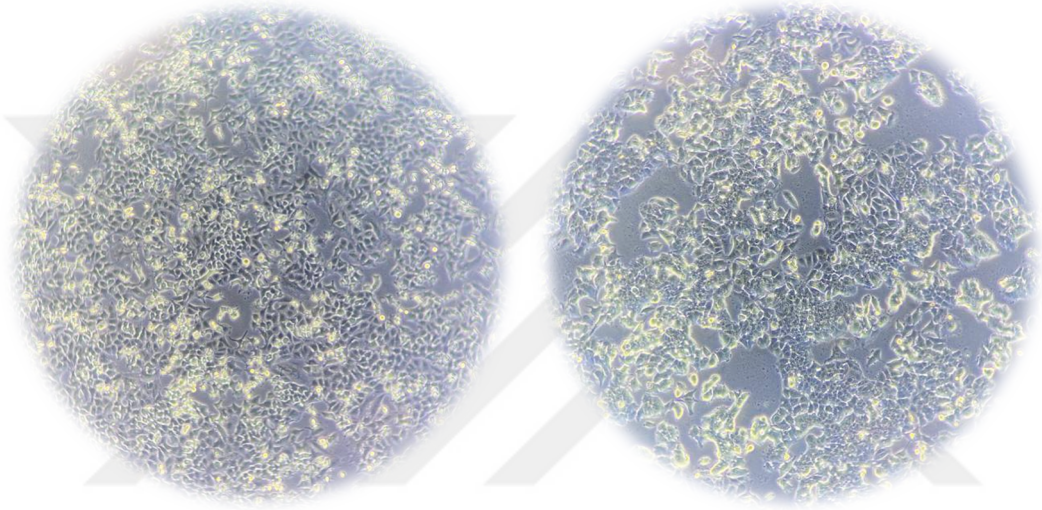


Figure 3.14 X-Ray Diffraction analysis result of polar microalgae

3.4 Anti-Cancer Activity

3.4.1 Cell Viablity – XTT Assay

In order to examine the anticancer effects of polar microalgae extracts and polar microalgal extract-loaded nanoparticles obtained within the scope of this thesis, first of all, cell lines were grown and their confuency was observed under the microscope (Figure 3.15).

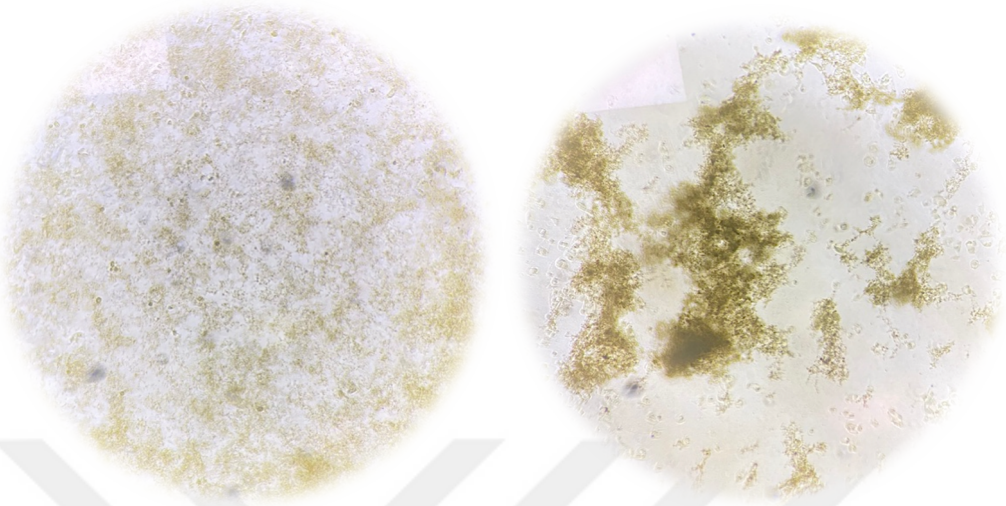


(A) **(B)**

Figure 3.15 A549 lung cancer cell line **(A)** and MCF-7 breast cancer cell line **(B)** under an inverted microscope (10x)

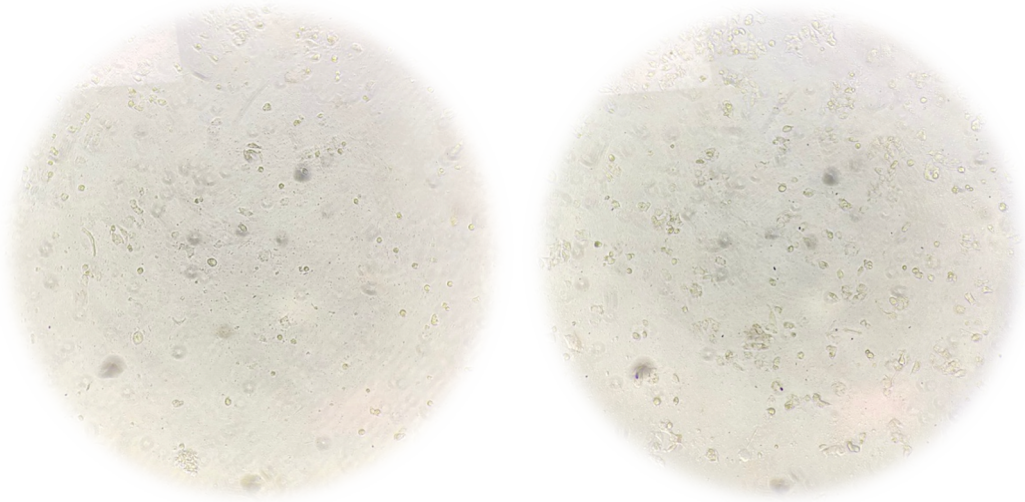
Then, XTT test was applied for the anticancer activity experiment. In this context, MCF-7 and A549 cancer cell lines were treated with samples (Figure 3.16 and 3.17) at different concentrations. At the same time, in order to observe the time-dependent effect, the samples were exposed for 24 h and 48 h in separate experimental setups, and the results obtained were determined by taking absorbance measurements with the help of a multiplate reader. Cell viability values were normalized according to cell viability grown in a sample-free medium. As a conctrol sample for polar microlag extracts DMSO and cell-free medium was used while for nanoparticles loaded with polar microalgae extract, it consists of PVA-AL coated nanoparticle without extract and cell-free medium used as a control. The results were performed

in triplicate at two different time intervals and the data obtained are consist of ± standard deviation values (Figure 3.18 to 3.21).



(A) **(B)**

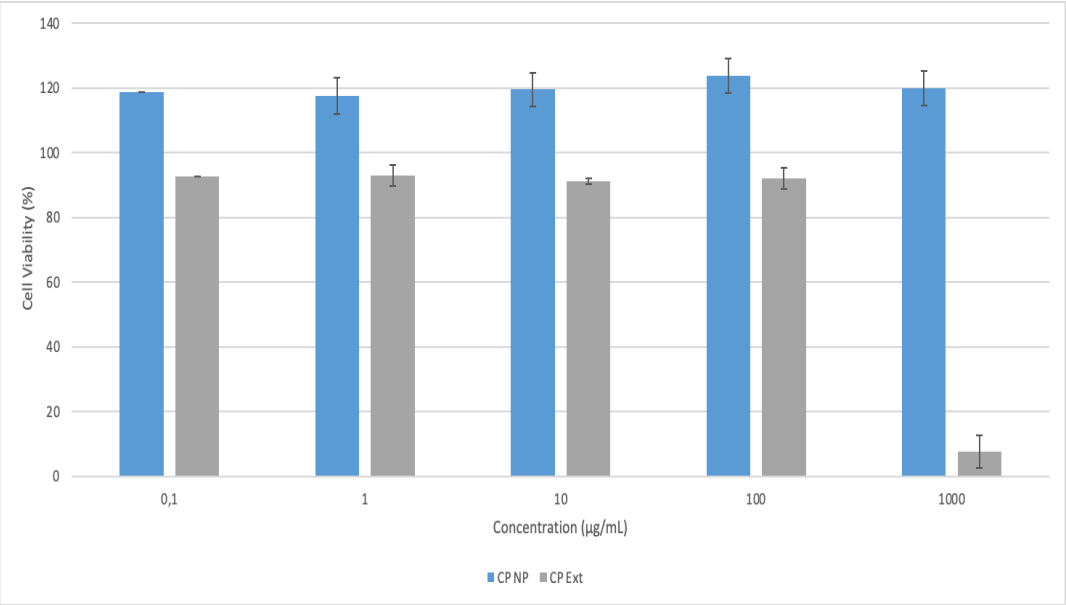
Figure 3.16 CP **(A)** and CS **(B)** extracts exposed to XTT plates for anticancer activity assay (10x)



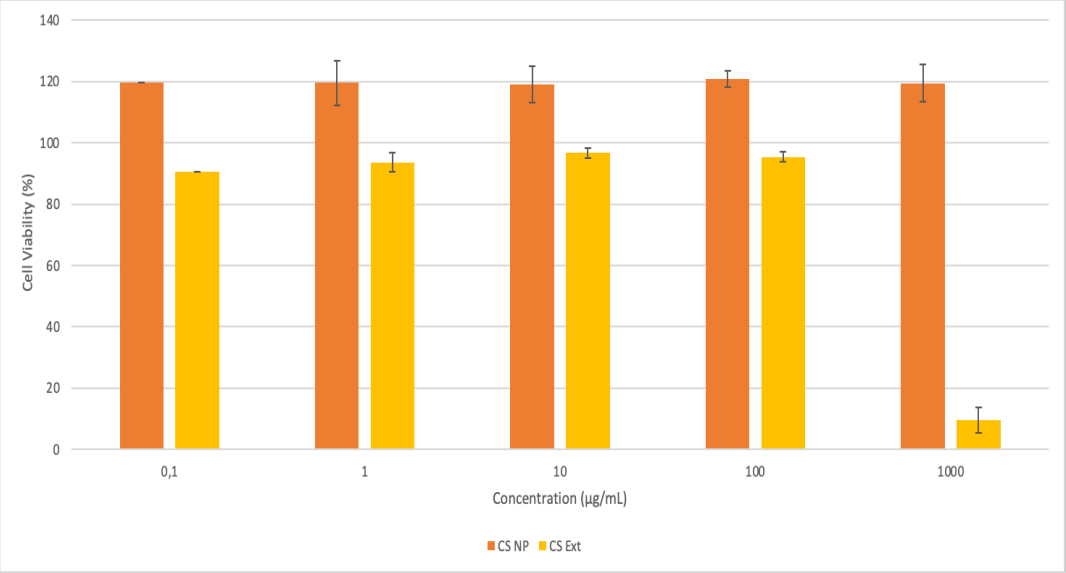
(A) **(B)**

Figure 3.17 CP loaded NPs **(A)** and CS loaded NPs **(B)** exposed to XTT plates for anticancer activity assay (10x)

The data obtained in line with this study show us that polar microalgae extracts and polar microalgal extract-loaded nanoparticles have anticancer effects, but further studies are needed. When all time-dependent graphs and graphs related to different cancer cell lines were examined, it was observed that the anticancer results obtained from the extracts were more effective than those obtained from the nanoparticles. Also, when the time-dependent graphs are examined in detail, it has been observed that as the exposure time of the samples increases, they show a better effect and decrease the cell viability more. When the results of the A549 lung cancer cell line were examined, it was observed that the extracts showed approximately 90% viability for both species and no significant difference was observed. When the time-dependent graphs are examined, it is observed that the cell viability of CP extracts regressed to around 80% without any significant difference after 48 h exposure time. CP-loaded NPs showed a significant decrease in cell viability at 48 h exposure time. At the end of the 48 h exposure time of CS samples, a decrease in cell viability was observed, but less than that of CP species. When the MCF-7 breast cancer cell line results were examined, better data were obtained in the results treated with 48 h samples compared to the 24 h exposure time. Especially for both polar microalgae species, there is a serious decrease in cell viability due to the concentration change after 48 hours treatment of the extracts, but this is not the case with the nanoparticles obtained from these extracts. Therefore, further research is needed. In addition, the data obtained show us that the cancer cell line has great importance in the results of anticancer activity. According to the observed results, it was determined that polar microalgae extracts and polar microalgae extract loaded nanoparticles showed more anticancer activity in lung cancer cells than in breast cancer cells. In addition, it was observed that there were changes in the viability of these cells over time. The longer the exposure time of cells with samples, the greater the decrease in cell viability was seen.

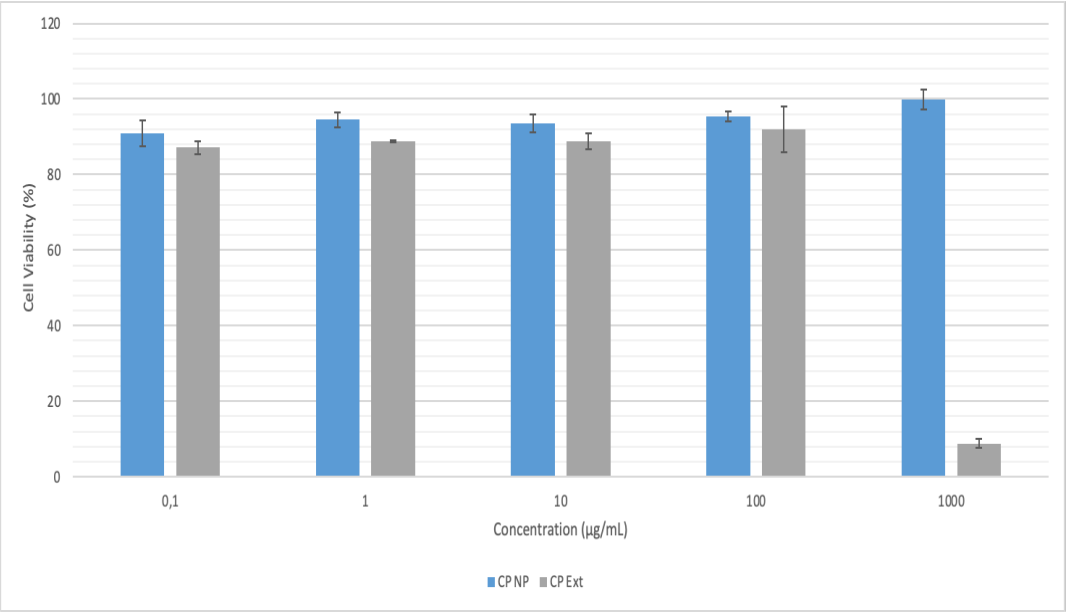


(A)

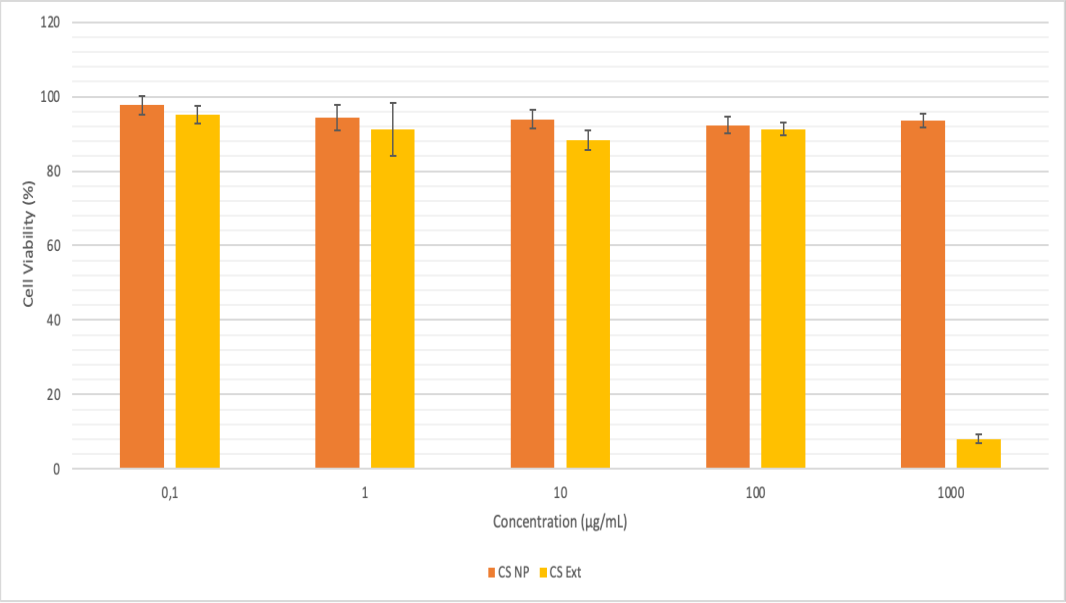


(B)

Figure 3.18 Cytotoxic effects of CP extracts and loaded NPs **(A)** and CS extracts and loaded NPs **(B)** on A549 lung cancer cell line with 24 h incubation period

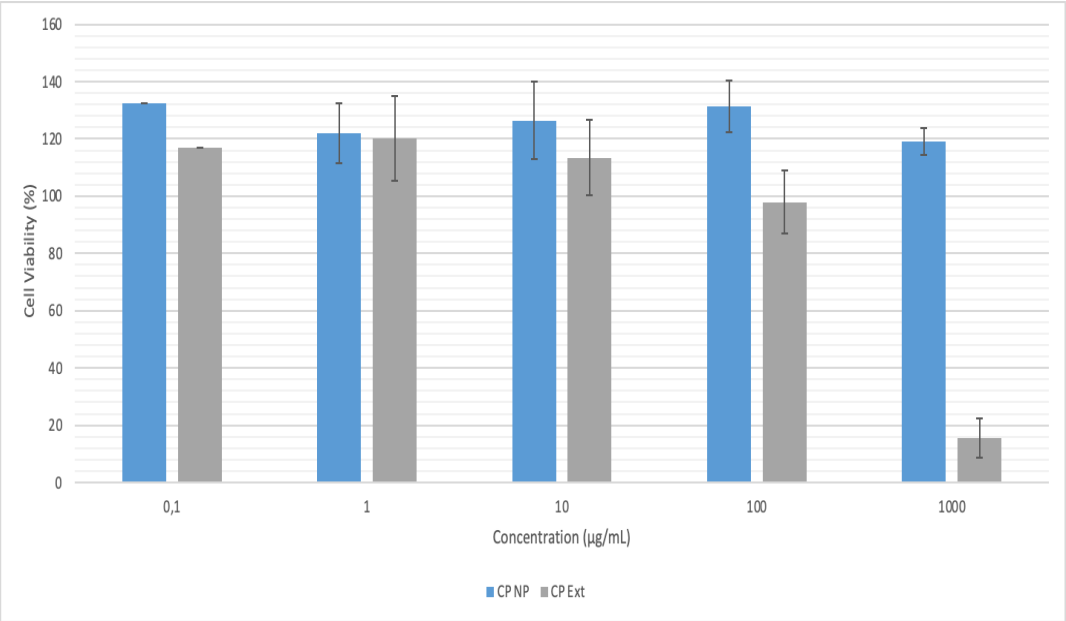


(A)

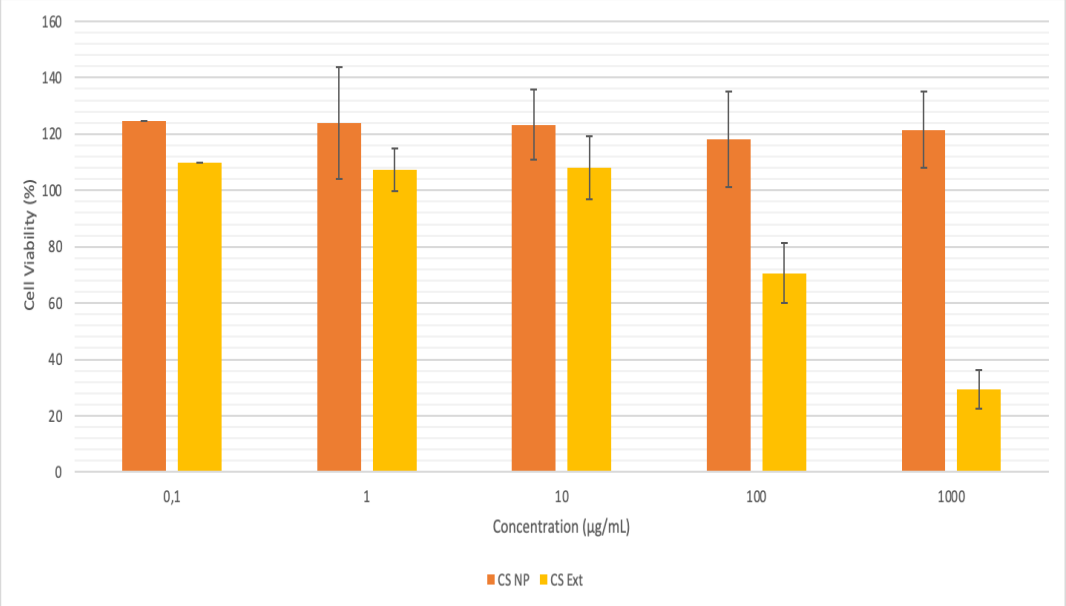


(B)

Figure 3.19 Cytotoxic effects of CP extracts and loaded NPs **(A)** and CS extracts and loaded NPs **(B)** on A549 lung cancer cell line with 48 h incubation period

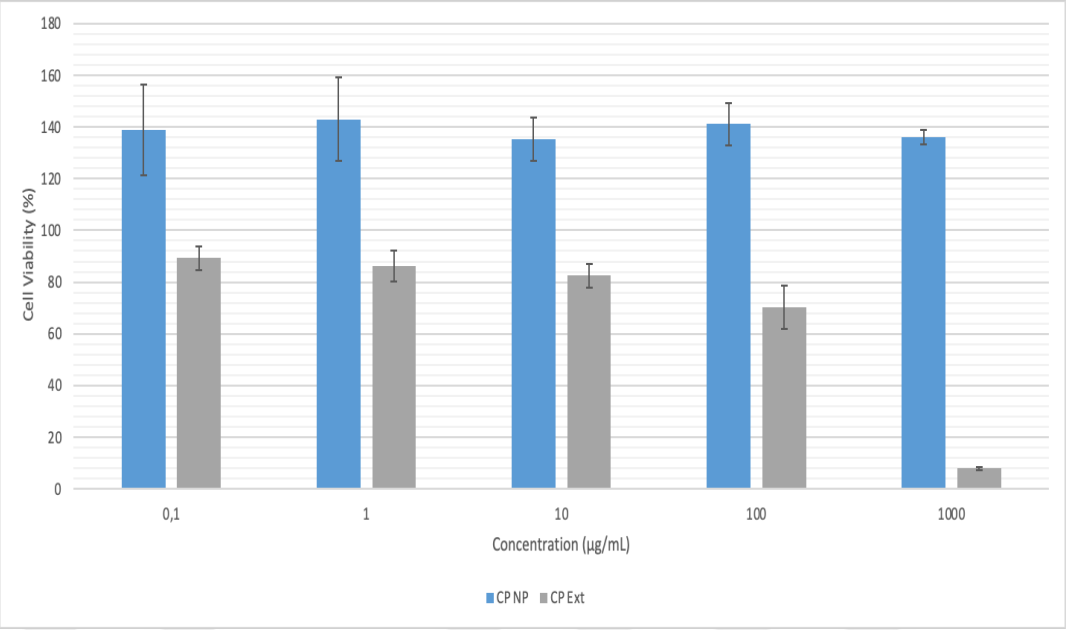


(A)

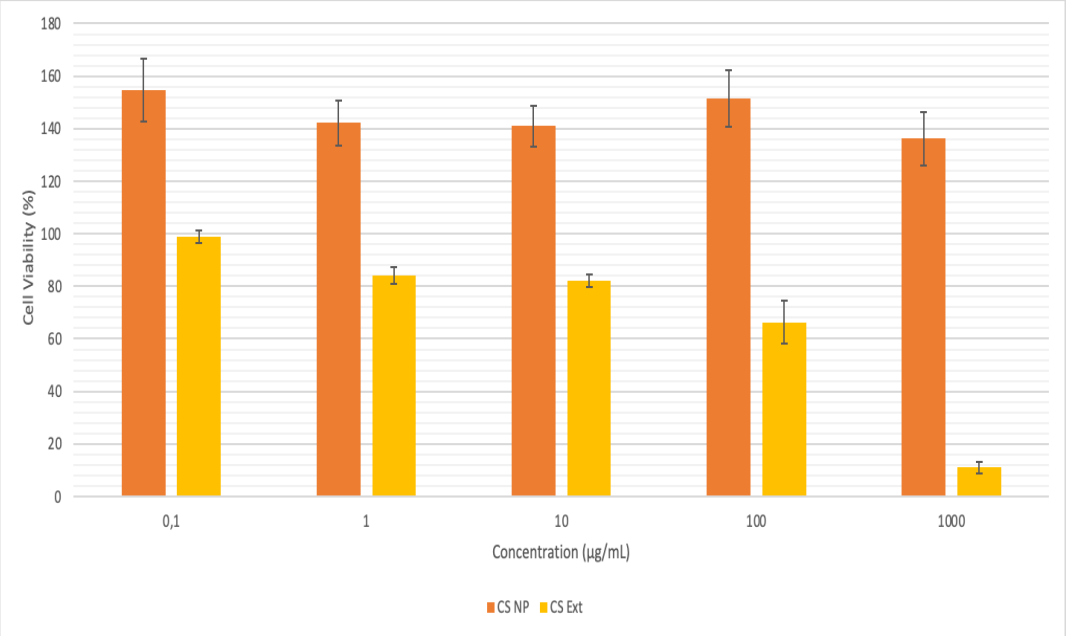


(B)

Figure 3.20 Cytotoxic effects of CP extracts and loaded NPs **(A)** and CS extracts and loaded NPs **(B)** on MCF7 breast cancer cell line with 24 h incubation period



(A)



(B)

Figure 3.21 Cytotoxic effects of CP extracts and loaded NPs **(A)** and CS extracts and loaded NPs **(B)** on MCF7 breast cancer cell line with 48 h incubation period

Studies in the literature on the anti-cancer activity of microalgae are limited. Some of these are aimed at investigating the effects of bioactive components in microalgae on anticancer activity and showing its use as a potential natural therapeutic product (Abd El-Hack et al., 2019; Martínez Andrade et al., 2018).

Karakaş et al. obtained nanoparticles by encapsulating *Chlorella protothecoides* and *Nannochloropsis oculata* microalgal extracts. In line with this study, they investigated the cytotoxic effect of microalgae extract loaded nanoparticles obtained at optimum processing parameters on brain glioblastoma and colon colorectal carcinoma cells and found promising results for cytotoxic effect on cancer cells (Karakaş et al., 2019).

Abolhasani et al., investigated the cytotoxic activity on the *Picochlorum* microalgae species extracted with different solvents in different cancer cell lines. They used MTT for cell viability testing and performed comparative analysis by growing cells in two dimensions and three dimensions, then apoptosis effects were examined. The results showed anti-proliferative activity in all cancer types and did not show significant activity in fibroblast cells. Also, it has been shown that apoptosis is induced as a result of the analysis. In addition, high level ROS production and MMP reduction were observed in some cell lines (Abolhasani et al., 2018).

In the study of Zhong et al., *Spirulina platensis* microalgae was loaded with chemotherapeutic doxorubicin and targated delivery was performed on lung metastasis of breast cancer cells. As a result of the study, ultrahigh drug loading efficiency and a controlled release were achieved, and this method was presented as an alternative to targeted drug delivery system (Zhong et al., 2020).

In another study, Alateyah et al. examined the effect of *Haematococcus pluvialis* strain on MDA-MB-231 breast cancer cells and observed that the strain showed anticancer activity. It has also been observed that inducing apoptosis occurs in line with some signaling pathways (Alateyah et al., 2022).

In the study conducted by Wali et al., the antioxidant, antibacterial, and antifungal and anticancer activities of the *Nannochloropsis oculata* microalgae extract were examined. Anticancer activity was tested on MDA-MB-231 human breast cancer

cells line and decreased viability was observed at increasing concentrations (Wali et al., 2020).

In a study by Lin et al., it was observed that *Chlorella sorokiniana* microalgae induced mitochondrial-mediated apoptosis through different mechanisms on non-small cell lung cancer cells (P.-Y. Lin et al., 2017).

In the study conducted by Elkhateeb et al., the anti-cancer activity of four different microalgae species on MCF-7 human breast cancer cells was investigated. The results showed that the highest cytotoxicity was observed in *Spirulina maxima* microalgae as 75.50±1.76 (Elkhateeb et al., 2020).

Kilari & Balakrishnan used *Chlorochromonas danica* in their study and examined the phytochemicals screenin and anti-proliferative activity on many cancer types. Different results have been obtained on different types of cancer. However, as a result of the study, an alternative approach that can be used in cancer treatment was presented (Kilari & Balakrishnan, 2023).

Reyna-Martinez et al. used lymphoma cell line and normal lymphocyte cells in their study. Antitumor activity and apoptosis analysis of *Chlorella sorokiniana* microalgae was performed on this cancer type and the results suggested that it showed anti-tumor activity (Reyna-Martinez et al., 2018).

In the studies conducted by Elia et al. with *Laurencia papillosa* microalgae, besides the cytotoxic effect, apoptosis determination, Western Blot and RT-qPCR analyzes were also examined. In the results, it was observed that the microalgae species had an anti-apoptotic effect (Elia et al., 2020).

In the study carried out by Inan et al., *Botryococcus braunii* and *Microcystis aeruginosa* microalgae and nanoparticles loaded with these microalgae were used. The results obtained did not show any cytotoxic effect in L929 normal cell line while, they showed different cytotoxicity results on different cancer types. However, it has been suggested that these microalgae species can be used in cancer treatment (İnan et al., 2021).

In the study carried out by Suh et al., the antioxidant, anti-proliferative, apoptotic and anti-invasive properties of the *Botryidiopsidaceae* polar microalgae species were investigated, and it was suggested that the microalgae species used in line with the results could be as a product for potential cancer treatment (Suh, Kim, et al., 2017).

Suh et al. investigated the anti-inflammatory and anticancer activities of Antarctic freshwater microalgae *Micractinium sp.* They suggest that it will show promising results in the treatment of coleractal cancer (Suh et al., 2018). In another study, it was observed that *Chloromonas reticulata* exhibited similar cytotoxic activity on HCT116 human colon cancer cell and provided a great reduction in the proliferation of cancer cells. They also suggested that there is an interaction between inflammation and cancer in both studies (Suh et al., 2019).

In another study by Suh et al., investigating molecular mechanisms of anticancer activity of Antarctic freshwater microalgae, *Chloromonas sp*. In line with the results, it was observed that it showed anti-proliferative effect on cancer cell lines and induced apoptosis with different pathways, while normal cells showed no anti-proliferative effect (Suh, Yang, et al., 2017).

In the study of Inan et al., *Chlorella variabilis* and *Auxenochlorella pyrenoidosa* polar microalgae and nanoparticles obtained from these microalgae were used, and their anti-cancer activities were compared on two different cancer cell lines. In line with the obtained results, polar microag extracts showed cytotoxicity around 50%. *C. variabilis* loaded nanoparticles showed better results than the other type of nanoparticle, although the results of cytotoxicity depending on the changing concentration (İnan, Mutlu, et al., 2023).

Bioactive components in the content of microalgae (İnan, Mutlu, et al., 2023), bioactive peptides (Skjånes et al., 2021) or high antioxidant activity of microalgae may be associated with anticancer activity (Shanab et al., 2012). Many active substances in its content can also cause anticancer activity by affecting the different signalling pathways (Bechelli et al., 2011; Haq et al., 2019). In addition, as observed in this thesis, flavonoids are thought to be a component related to anticancer activity (Ekalu & Habila, 2020; Ferdous & Balia Yusof, 2021). In this direction, the reason

for the anticancer activity of microalgae and the underlying molecular mechanisms and signal pathways should be investigated. In addition, further studies are needed for active substance research on the use of microalgae as a potential biotechnological drug in cancer treatment.

4
CONCLUSION

In line with this thesis, cultivation of CP and CS microalgae, extraction of the obtained microalgal biomass, biochemical content analysis, encapsulation of polar microalgae extracts with electrospraying method and detailed characterization of the obtained nanoparticles were carried out. However, the main aim of the thesis is investigation of the cytotoxic effects of polar microalgae extracts and PVA-AL coated nanoparticles obtained from these extracts on A549 lung cancer and MCF7 breast cancer cell lines with different time intervals. In this direction, the general findings obtained from this thesis study are summarized below:

- According to the growth kinetics calculations, specific growth rate values are found as 0.16 and 0.15, respectively for CP and CS polar microalgae. Also doubling time values are found as 4.33 and 4.62, respectively.
- According to the growth kinetics curves both polar microalgae species grew between 3 and 13 days, then reached the stationary phase, and lastly passed into the death phase.
- Carbonhydrate content of CP and CS polar microalgae were found to be 43.33 and 35.56%, respectively; and the protein contents were calculated as 30.58 and 28.06%, respectively.
- The amount of $\beta-carotene$ from CP and CS polar microalgae, was calculated as 9.11 and 6.14 µg/mL, respectively; and the chlorophyll-a contents were calculated as 6.14 and 11.33 µg/mL, recpectively.
- As a result of thermogravimetric analysis of CP species, moisture, volatile matter, fixed carbon, and ash contents were determined as 10.23%, 64.33%, 19.71% and 5.73%, respectively. While CS species, moisture, volatile matter, fixed carbon, and ash contents were determined as 7.9%, 74.03%, 11.93% and 6.14%, respectively.

- The functional groups were determined with FTIR analysis. Each peak assigned a functional group. These bands in these spectrums indicates lipid, carbohydrate, protein groups, which were also observed in previous results with polar microalgae extracts, are observed and these bands overlap. This overlap indicates the presence of oil in the content of the produced nanoparticles. Therefore, it can be said that polar microalgae extract is encapsulated in this direction. The data in the literature also confirms this.
- The lipid content of CP and CS microalgae was calculated as 14 and 27% DW, respectively.
- It was discovered that the microalgae extracts used in this study showed antioxidant capabilities by having 38% radical scavenging activity. In addition, the antioxidant activities of the microalgae species used in the study were observed to be similar to each other.
- The phenolic contents of CP and CS polar microalgae extracts were calculated as 43.19 mg GAE/g DW and 42.04 mg GAE/g DW, respectively. The flavonoid contents of CP and CS polar microalgae extracts were calculated as 1.16 mg Q/g DE and 0.6 mg Q/g DE, respectively. The Antarctic microalgae species was discovered to have a greater flavonoid content than the other polar species.
- The morphological properties of polar microalgae extracts-loaded nanoparticles are determined at 25, 100 and 250 kX magnification by FESEM analysis. In accordance with the FESEM analysis results, it was observed that the nanoparticles were in a spherical shape.
- The average diameter of the nanoparticles loaded with CP was calculated as 294.7 nm and the PdI value as 0.406; the average diameter of the nanoparticles loaded with CS microalgae extract was calculated as 156.4 nm and 0.401 in PdI value. These findings demonstrated that the generated nanoparticles were of sufficient size to be considered as nanoparticles.
- FTIR analysis of nanoparticles showed that the oil of polar microalgae was encapsulated successfully and the bands which related to lipids of the microalgae oil and microalgae oil loaded nanoparticles were overlapped.

- The encapsulation efficiencies of nanoparticles loaded with CP and CS extracts produced as a result of the thesis study were determined to be 85% and 92%, respectively. This demonstrates that the encapsulation procedure has been carried out effectively and provides data to validate the findings of other analysis results.
- The *in vitro* release experiment of nanoparticles shows that the encapsulated bioactive will be effectively released within the first 24 hours. Also, this oscillation happened gradually, rather than abruptly. In this approach, it has been seen that the nanoparticle obtained in the study will be released in a controlled manner in the body when utilized as a potential biotechnological drug in future investigations.
- When the XTT results of the A549 lung cancer cell line were examined, it was observed that the extracts showed approximately 90% viability for both species and no significant difference was observed. When the time-dependent graphs are examined, it is observed that the cell viability of CP extracts regressed to around 80% without any significant difference after 48 h exposure time. CP loaded NPs showed a significant decrease in cell viability at 48 h exposure time. At the end of the 48h exposure time of CS samples, a decrease in cell viability was observed, but less than that of CP species.
- When the MCF-7 breast cancer cell line results were examined, better data were obtained in the results treated with 48 h samples compared to the 24 h exposure time. Especially for both polar microalgae species, there is a serious decrease in cell viability due to the concentration change after 48 hours treatment of the extracts, but this is not the case with the nanoparticles obtained from these extracts.
- It was observed that the anticancer activity of the extracts were more effective than the nanoparticles. It was determined that polar microalgae extracts, and polar microalgae extract loaded nanoparticles showed more anticancer activity in lung cancer cells than in breast cancer cells. In addition, it was observed that, there were changes in the viability of these

cells over time. The longer the exposure time of cells with samples, the greater the decrease in cell viability was seen.

For future studies, evaluating the change in flavonoid contents of polar microalgae grown under different cultivation conditions and its effect on various cancer cell lines are crucial. In this direction, optimization of the cytotoxicity analysis under different conditions can be needed for further studies. In addition, advanced cancer research, investigation of apoptotic effects, cell cycle analysis, molecular mechanisms and signaling pathways that affect anticancer activity should be carried out in order to develop drug formulations. In this way, a potential alternative biotechnological drug candidates for cancer can be obtained.

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PUBLICATIONS FROM THE THESIS

Conference Papers

1. İnan, B., Mutlu, B., Karaca, G. A., Koç, R. Ç., & Özçimen, D. (2022). Cultivation of Chlorella variabilis YTU.ANTARCTIC.001 and Evaluating Its Anticancer Activity against PC-3 and AGS Cell Lines. III. International Enzyme and Bioprocess Days EBDays 2022, Turkey, 9

Papers

1. İnan, B., Mutlu, B., Karaca, G. A., Koç, R. Ç., & Özçimen, D. (2023). Bioprospecting Antarctic microalgae as anticancer agent against PC-3 and AGS cell lines. *Biochemical Engineering Journal*, *195*, 108900. https://doi.org/10.1016/j.bej.2023.108900