TÜRKİYE CUMHURİYETİ
ADANA ALPARSLAN TÜRKEŞ SCIENCE AND TECHNOLOGY UNIVERSITY

GRADUATE SCHOOL
BIOENGINEERING DEPARTMENT

DETERMINATION OF POTENTIAL ANTI-CANCER EFFECT OF LOCALLY GROWN *ARUM DIOSCORIDIS* L. PLANTS

UKBA TURAP OĞUR

M.Sc.

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THESIS ADVISOR
ASSIST. PROF. DR. ÖZGÜR CEM ERKİN

ADANA, 2024

DECLARATION OF CONFORMITY

In this thesis study, which was prepared following the thesis writing rules of Adana Alparslan Turkes Science and Technology University Institute of Graduate School, I declare that I provide all the information, documents, evaluations and results in accordance with scientific ethics and moral codes without resorting to any means or assistance that would be contrary to scientific ethics and traditions. I also declare that I refer to all of the articles I used in this study with appropriate references and accept all moral and legal consequences if a situation is found contrary to my statement regarding my work.

13/11/2024

[Signature]

Ukba Turap OĞUR

ÖZET

YERELDE YETİŞTİRİLEN *ARUM DİOSCORİDİS* L. BİTKİLERİNİN POTANSİYEL ANTİ-KANSER ETKİSİNİN BELİRLENMESİ

UKBA TURAP OĞUR
Yüksek Lisans, Biyomühendislik Anabilim Dalı

Danışman: Dr. Öğr. Üyesi Özgür Cem ERKİN
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Kanser hastalıkları hem gelişmiş hem de gelişmekte olan ülkelerde önemli bir sağlık sorunudur. Dünya çapında her yıl milyonlarca insana kanser teşhisi konulmakta ve teşhis edilen hastaların pek çoğu hayatını yitirmektedir. Kansere bağlı ölümler arasında birinci sırada bulunan akciğer kanseri, ikinci sıradaki kolorektal kanserden (CRC) 2,5 kat daha fazla görülme sıklığına sahiptir. Akciğer kanseri, küçük hücreli akciğer kanseri ve küçük hücreli dışı akciğer kanseri olmak üzere iki farklı alt gruba ayrılmakta, vakaların yaklaşık %85'ini ise küçük hücreli dışı grubu oluşturmaktadır. Kanser hastalığının ilerlemesini durdurmak, tedavisini ve kontrolünü geliştirmek için son zamanlarda pek çok araştırma yapılmış olmasına rağmen, hali hazırda yenilikçi ve alternatif yaklaşımlara ihtiyaç duyulmaktadır. Örneğin, kanser tanısı alan birçok hasta, kemoterapi ve radyasyon tedavisinin ciddi yan etkileri nedeniyle alternatif veya tamamlayıcı doğal bileşenli tedavi yöntemleri aramaktadır. Kanser önleyici sekonder metabolitlere sahip birçok bitki vardır. *İn vitro* çalışmalar, bitki ikincil metabolitlerinin çeşitli kanser mekanizmaları üzerinde potansiyel etkileri olduğunu göstermektedir. Ülkemizde Adana başta olmak üzere çeşitli bölgelerde yabani olarak yetişen ve halk arasında gıda olarak tüketilemesinin yanı sıra, geleneksel tıpta da kullanılan *Aracea* familyasına ait *Arum* cinsi *Arum dioscoridis* L. bitkisi, bazı çalışmalarda kullanılan ve anti-kanser etkileri olduğu araştırmalarca belirlenmiş, tedavi edici bitkiler arasındadır.

Bu çalışmada *Arum dioscoridis* L. bitkisi tohum özütünün küçük hücreli dışı bir akciğer kanseri hücre hattı olan A549 üzerindeki, potansiyel antikanser etkileri araştırılmıştır. Bu amaçla bitkinin tohumları kurutulup öğütülerek, eşit miktarda metanol ve su içeren bir çözgen

ile özüt hazırlanmıştır. Bitki özütünün potansiyel sitotoksik etkilerini belirlemek amacıyla A549 (akciğer), HUH-7 (karaciğer), MCF-7 (göğüs) kanser ve BEAS-2B (akciğer-normal) hücre hatları üzerinde MTT hücre canlılık testi uygulanarak özütün farklı konsantrasyonlarda hücreler üzerinde öldürücü etkiye sahip olduğu tespit edilmiştir. A549 hücre hattında hedeflenen hücre canlılığının azalması sonucunda çalışmanın geri kalan kısımlarına A549 hücre hattı ile devam edilmiştir. A549 hücreleri üzerinde yara iyileştirme testi ile özütün hücrelerin göç ve istila yetenekleri üzerindeki potansiyel etkileri gözlemlenmiştir. Özütün, hücrelerin agresif yayılımının bir göstergesi olan koloni oluşturma kapasiteleri üzerindeki etkileri yumuşak agar koloni oluşturma testi yapılarak; apoptoz tetikleme potansiyeli ise DAPI, AnnexinV- PI boyama ile belirlenmiştir. Son olarak etkili bir anti karsinojen ajanın önemli bir özelliği olan hücre döngüsünü durdurma potansiyeli ise akış sitometrisi yöntemi ile analiz edilmiştir.

Analiz sonuçlarına göre, hidrometanolik çözgen ile elde edilen *Arum dioscoridis* L. tohum özütünün, konsantrasyona bağlı olarak A549, HUH-7, MCF-7 ve BEAS-2B hücrelerinin canlılığını azalttığı belirlenmiştir. Bunun yanısıra elde edilen özütün konsantrasyon artışı ile A549 hücrelerinde göç yeteneğini ve koloni oluşumunu engellediği, ayrıca hücrelerin hücre döngüsünü bloke etmediği, ancak apoptozu tetiklediği görülmüştür. Yapılan araştırma sonucunda edinilen bulgular, *Arum dioscoridis* L. bitki tohumlarının küçük hücreli olmayan akciğer kanseri tedavisi için yeni bir alternatif terapötik kaynağı olabileceğini göstermektedir.

Anahtar Kelimeler: Küçük hücreli dışı akciğer kanseri, A549 hücre hattı, *Arum dioscoridis* L., anti-cancer etki, hidrometanolik tohum özütü.

ABSTRACT

DETERMINATION OF POTENTIAL ANTI-CANCER EFFECT OF LOCALLY GROWN *ARUM DIOSCORIDIS* L. PLANTS

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In both industrialized and developing nations, cancer is a significant health concern. Millions of people around the world are diagnosed with cancer every year, and many of them loose their life. Lung cancer, which is the top cause of the cancer-related deaths, is 2.5 times more common than colorectal cancer (CRC), which ranks second. Lung cancer is divided into two categories: small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). NSCLC accounts for 85% of all lung cancer cases. Although much research has been done recently to advance the treatment and control of cancer disease progression, significant work and improvements can still be made. Because of the severe side effects of radiation and chemotherapy, many cancer patients are searching for complementary or alternative treatment approaches that use natural components. There are many plants with secondary metabolites having anti-cancer effects. Plant metabolites contribute to their anti-cancer nature in various ways, and *in vitro* studies demonstrate the potential of secondary metabolites in anti-cancer action. *Arum dioscoridis* L. plant of the *Arum* genus belong to the *Aracea* family, which grows wild in various regions of our country and consumed as food by the public especially in Adana. Also used in traditional medicine, is among the therapeutic plants used in many studies and determined by research to have anticancer effects.

In this study, the anticancer effects of *Arum dioscoridis* L. plant on lung cancer A549 cell line were investigated. *Arum dioscoridis* L. plant seeds were dried, ground and extracted with MeOH + H_2O solvent. The cytotoxic effect of *Arum dioscoridis* L. extract at different concentrations on A549, HUH-7, MCF-7, BEAS-2B cell lines was determined to have a

cytotoxic effect on the cells by performing the MTT cell viability test. As a result of the decrease in the targeted cell viability in the A549 cell line, the experiments were continued with the A549 cell line. The effects on the migration abilities of the cells were observed with the wound healing test on A549 cells; the effects of the cells on colony formation were determined by performing the soft agar colony formation test; the effects on apoptosis were determined by DAPI, AnnexinV-PI staining and the effects on the cell cycle were determined by performing Facs analysis.

According to the analysis results, it was determined that Arum dioscoridis L. extract formed with MeOH + H_2O (hydromethanolic) solvent decreased A549, HUH-7, MCF-7, BEAS-2B cells viability. It was observed that hydromethanolic ic plant extract A549 cells prevented migration ability and colony formation as the concentration increased and also didn't block the cell cycle in A549 cells but triggered apoptosis. The findings obtained as a result of the research conducted suggest that Arum dioscoridis L. plant may be a new alternative therapeutic source for the treatment of non-small cell lung cancer.

Keywords: Non-small cell lung cancer, A549 cell line, *Arum dioscoridis* L., anti-cancer, hydromethanolic plant extract

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TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

LIST OF ABBREVIATIONS

NSCLC	: Non-small cell lung cancer
SCLC	: Small cell lung cancer
CRC	: Colorectal cancer
SCC	: Squamous cell carcinoma
EGFR	: Epidermal growth factor receptor
KRAS	: Kristen rat sarcoma
ALK	: Anoplastic lymphoma kinase
WHO	: World health organization
NCI	: National cancer institute
DNA	: Deoxyribonucleic acid
MTT	: 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide
EGCG	: Epigallocatechin gallate
FDA	: Food and drug administration
A549	: Lung cancer
CFPAC-1	: Pancreatic cancer
MeOH	: Methanol
HMPE (1:1)	: Hydroxymethanolic plant extract (1:1)
Dh20	: Deionized water
FBS	: Fetal bovine serum
PS	: penicillin streptomycin
PBS	: Phosphate buffered saline
CO2	: Carbondioxide
TM	: Treatment medium
RPMI	: Roswell Park Memorial Institute
EDTA	: Etilendiamin Tetraasetic Acid
DMEM	: Dulbecco's Modified Eagle Medium
IC50	: Half maximal inhibitory concentration

1. INTRODUCTION

Cancer is a disease that can develop in over 100 different cell types, tissues and organs, characterized by uncontrolled cell proliferation. Mechanisms that control the proliferation, differentiation and survival represent abnormalities in cancer cells. The uncontrolled growth and spread of these abnormal cells result in a series of diseases known as cancer (Ozturk, 2017). Cancer is among the important social, public health and economic problems of the 21st century. Cancer disease, which causes three out of every 10 premature deaths caused by non-communicable diseases (30.3% of those in the 30-69 age group), is among the three most important causes of death in this age group in 177 of 183 countries (Bray et al., 2024).

It is recorded in the statistics made by GLOBOCAN that the number of patients diagnosed with lung cancer in the world is more than 2.2 million and there are approximately 1.8 million deaths. According to these statistics, lung cancer is recognized as the leading cause of cancer-related fatalities worldwide (Jachowski et al., 2023). Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are the two types of lung cancer (Nakamura, 2022). Eighty five percent of all instances of lung cancer are NSCLCs (Veronesi, 2015).

It is essential today to identify new, alternative treatment agents in the intervention of fatal NSCLC. The search for alternatives has arisen due to the decrease in the effects of treatment methods and the increase in side effects experienced by the patient due to the drugs used in the treatments. Recently, many researchers have been investigating natural products, especially plant-derived compounds, that can be used instead of or in combination with synthetic compounds used in treatments, due to their potential antitumor properties (Zhang et al., 2018).

The National Cancer Institute (NCI) of United States of America studied nearly 35,000 plant species with potentially anti-cancer properties. Among them, approximately 3,000 plant species have shown ongoing anticancer properties (Desai et al., 2008). Many plants contain products that have been shown to be effective in treating cancer. This effect occurs by inhibiting cancer-activating enzymes, stimulating the DNA repair mechanism, inducing an antioxidant effect and promoting the production of protective enzymes (Bharadvaja, 2017).

Arum dioscoridis L. plant of the *Arum* genus belonging to the *Aracea* family is a plant species that has been used among locals for centuries (Uçan Türkmen et al., 2019). In Anatolia, it is

consumed as a herbal medicine against shortness of breath, asthma, bronchitis, chest softening, expectoration, intestinal laziness, digestive system, mumps, serpentine disease, bladder and urinary tract diseases, malaria and especially hemorrhoids (Değer, 2022).

A study utilizing the GC-MS method analyzed the extract of locally grown *Arum dioscoridis* L. plants, revealing 16 distinct chemicals of pharmacological and medicinal significance in the MeOH ic extract. Due to its antioxidant characteristics and a significant concentration of phenolic compounds, *Arum dioscoridis* L. plant extract was suggested as a promising candidate for cancer research (Yabalak, 2018). Altough there is enough evidence, studies aiming to characterize chemotherapoutic potential of this plant is rather limited in our country. Therefore it was choosen as an alternative source of study material in this thesis.

The main aim of this study is to investigate the potential anti-cancer effects of hydromethanolic ic extracts of *Arum dioscoridis* L. seeds on various cancer cell lines. Specifically, the plant extract was used to determine potential changes in cell viability, migration ability, colony forming capacity and cell cycle progression by various *in vitro* methods on A549 NSCLC cell line.

2. LITERATURE REVIEW

2.1. Cancer

Abnormal cell growth due to genetic or epigenetic changes in somatic cells that can spread to other parts of the body is known as cancer (Saini et al., 2020). It is a disease that can develop in over 100 different cell types, tissues, and organs, characterized by uncontrolled cell proliferation or tumor formation. Mechanisms that control the proliferation, differentiation, and survival of cells represent abnormality in cancer cells. Loss of apoptotic functions results in further uncontrolled cell growth and differentiation and a neoplastic cell population, resulting in cancer progression.

The most important problem of cancer is the migration of the abnormally growing cells to the other parts of the human body. For instance skin wart, common in the human body, is a benign tumor. Its good condition means that it stays where it proliferates and does not damage the real tissue around it or not expands to other dimensions in the body. In contrast, a malignant tumor is aggressive. They can attack surrounding healthy tissues, invade the entire body and metastasize by using the tissue regenerative and lymphatic systems. Cancer and the diagnosis of the disease reveal the symptoms for the presence of malignant tumors in humans. The most important and dangerous ability of malignant tumors is their capacity to invade and metastasize. The treatment of benign tumors is sometimes not even done without medical intervention. On the contrary, malignant tumors show resistance to healing in the area (Cooper and Hausman, 2007).

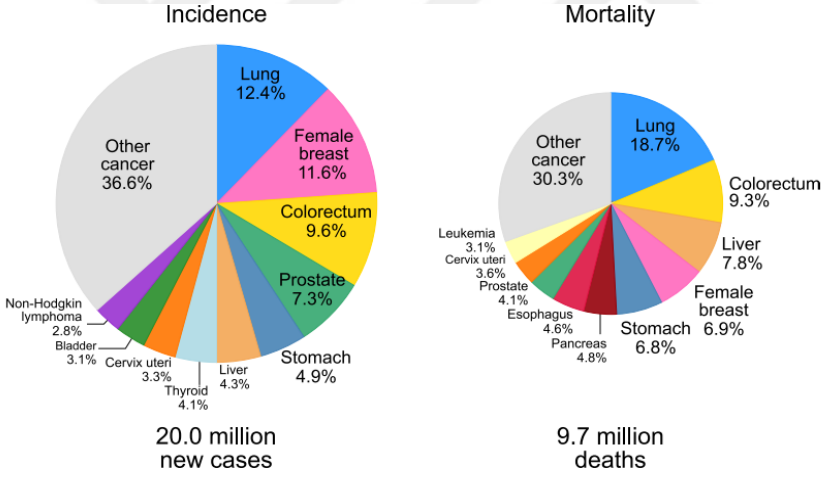
External causes of cancer are overexposure to radiation, ultraviolet rays, pollution, smoking and stress, as well as causes at the cellular level such as lack of apoptotic function, genetic mutations, oxidative stress and hypoxia (Abotaleb et al., 2019). The fact that early diagnosis methods of cancer are not widespread and comprehensive, patients diagnosed at an advanced stage and the incidence of cancer diagnoses continue to increase worldwide.

2.2. Prevalence of Cancer in the World

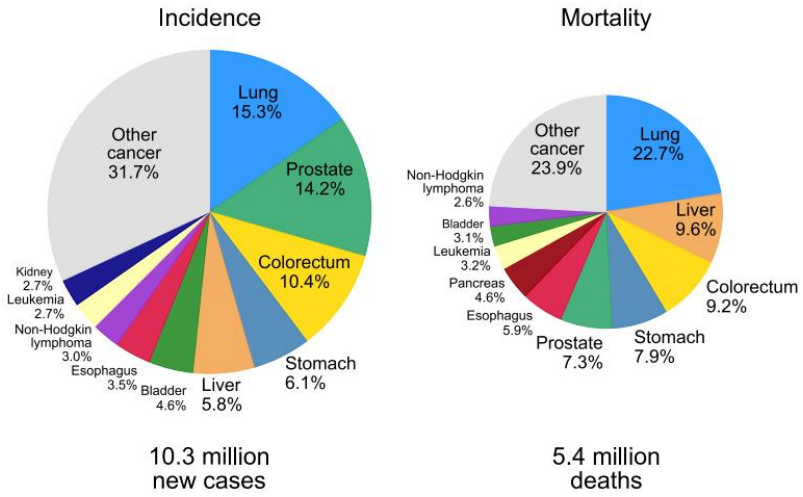
Epidemiological data show that almost one in six deaths (16.8 %) and one in four deaths (22.8 %) from non-communicable diseases worldwide are cancer (Bray et al., 2024). Global demographics predict that by 2025, an average of 420 million new cancer diagnoses will

occur each year. In line with these predictions, it can be concluded that the diagnosis of cancer will increase every year (Saini et al., 2020). Cancer is among the important social, public health, and economic problems in the 21st century. Cancer disease, which causes three out of every 10 premature deaths caused by non-communicable diseases (30.3 % of those in the 30-69 age group), is among the three most important causes of death in this age group in 177 of 183 countries (Bray et al., 2024).

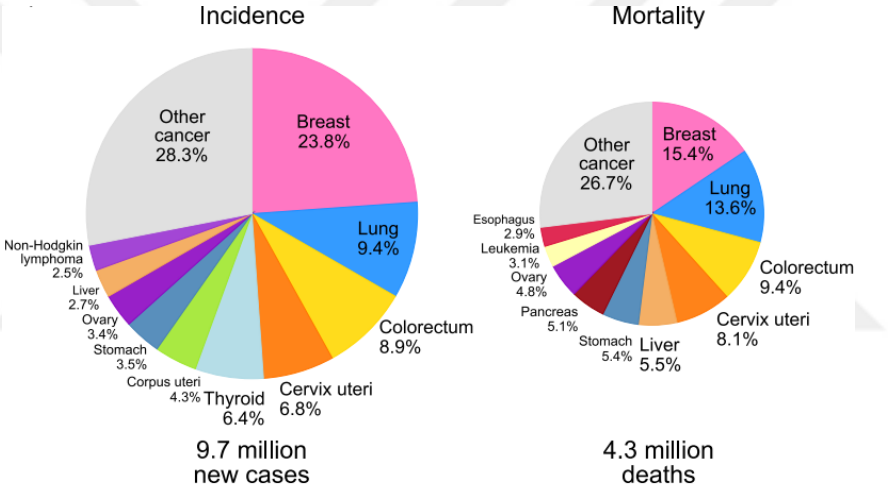
GLOBOCAN 2022 data, which represents the top 10 cancer types in cancer-related deaths in the world and the distribution of deaths from this disease are shown in Figure 2.1. For men and women, these 10 types of cancer account for more than 60 % of newly diagnosed diseases and deaths. Lung cancer is the most frequently diagnosed cancer in the world and has the highest mortality rate. The most common type of cancer in women is breast cancer. Lung cancer and breast cancer have become the leading causes of cancer-related deaths (Sung et al., 2021).



A) Both Genders



B) Males



C) Females

Figure 2.1. Shows the cancer-related incidence and death distributions of the top ten cancers for (A) both genders, (B) male, and (C) female. Source: Globocan2022

2.3. Lung Cancer

Lung cancer kills approximately 350 people every day. It is 2.5 times more common than colorectal cancer (CRC), which is the second leading cause of cancer-related deaths (Siegel et al., 2023). GLOBOCAN statistics show that the number of patients diagnosed with lung cancer in the world is more than 2.2 million and there are approximately 1.8 million deaths. In line with these numbers, it is unrstood that lung cancer is the most common cause of cancer deaths in the world (Jachowski et al., 2023). Of the 127,070 deaths in 2023, approximately

103,000 (81 %) were due to direct smoking and 3,560 were due to passive smoking. The remaining approximately 20,500 deaths from non-smoking lung cancer have been classified as the eighth leading cause of cancer death across genders (Siegel et al., 2023).

The most common risk factor for lung cancer development is smoking. Other risks include passive smoke breathing, residential ra, pollution and air quality, workplace exposures such as asbestos. Current cultural and regional differences in tobacco smoking between men and women affects disease appearance rates, yet, they are increasing globally. Moreover, lung cancer insidence rates increases among never-smokers as well as age of diagnosis decreases among different human populations.

2.3.1. Classification of lung cancer

The World Health Organization's (WHO) 2015 classification of lung cancers is the basis for determining the types of this disease (Clark and Alsubait, 2023). Lung cancer is divided into two categories: small-cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). (Nakamura, 2022). Immunohistochemistry and light microscopy are used in this classification approach to help predict a prognosis course and better guide treatment. The phrase "non-small cell lung cancer" (NSCLC) refers to a group of lung malignancies, including large cell, and squamous cell carcinomas, and adenocarcinoma (Clark and Alsubait, 2023).

NSCLC accounts for 85% of all lung cancer cases (Veronesi, 2015). Half of all cases of lung cancer are adenocarcinomas, which are the most prevalent type of lung cancer in this category. Before this point, the most common type of NSCLC to be diagnosed with lung cancer was squamous cell carcinoma (SCC). Other lung cancer subgroups that are both heterogeneous and have broad terminology are included in NSCLC. These are non-small cell neuroendocrine tumors, sarcomatoid and adenosquamous carcinomas (Clark and Alsubait, 2023). The mechanisms involved in the pathogenesis and treatment of NSCLC are complex. Common therapeutic options recommended for treatment after diagnosis of NSCLC include chemotherapy and surgery. These treatment methods have a partial effect for patients with advanced NSCLC (Zhang et al., 2018).

Lung tumor formation is influenced by certain mutations and gene changes. These changes are present in genes encoding proteins such as epidermal growth factor receptor (EGFR), Kirsten rat sarcoma virus (KRAS), anaplastic lymphoma kinase (ALK), B-RAF, and ROS1 (Jachowski et al., 2023).

The high mortality rate of NSCLC creates a great need for treatment and the development of more effective methods to cure the disease. Due to this need, new NSCLC treatment methods are starting to play a more important role. However, classical methods such as surgery, radiotherapy and chemotherapy are still widely used in clinical treatment worldwide. In addition to these treatments, ongoing research on alternative or collectively traditional treatments enables the disease to be cured and the treatments to be used more effectively (Jachowski et al., 2023)(R. Siegel et al., 2014).

2.4. Importance of Cancer Research

Cancer disease is an important health problem in both developed and developing countries. Millions of people are diagnosed with cancer every year around the world, and most of the diagnosed patients die. According to the American Cancer Society, 2-3% of annual deaths recorded worldwide are due to cancer.

Cancer is among the most resistant diseases in terms of treatment processes and treatment success globally. The mechanisms of cancer disease are complex and not fully understood, and cancer cells are very similar to healthy cells. Cancer cells can grow abnormally and cause the death of normal cells in the body. The reason why it causes the death of normal cells is because it invades and then destroys normal cells. Cancer cells are born due to some imbalances in the human body. When this imbalance within the body is corrected, cancer can be cured (Prakash et al., 2013).

Billions of dollars have been spent over the years on the treatment and research of a disease that is so common. Even with all this research, it is still not fully understood what cancer is. Many medications are available to treat various types of cancer. For example, several anti-chemotherapeutic agents are used, but when their use is reduced, toxicity occurs in the human body. Therefore, it cannot be said that any medicine is completely effective and safe.

Although much research has recently been done to advance the treatment and control of cancer disease progression, significant work and improvements can still be made (Ignacimuthu et al., 2006). To guide public health initiatives focused on cancer prevention, early cancer detection, and cancer treatment, it is critical to comprehend the prevalence of cancer. Current treatment methods currently neither cure nor significantly improve chances of

survival except in rare cases. As a result, new treatment agents and new targets must be determined (Li et al., 2009).

2.5. Cancer Treatment Types

A patient diagnosed with cancer has various treatment options depending on the type of cancer and its progression. Treatment methods are determined according to the patient's condition. Some patients may receive a single cancer treatment. However, most of the time, patients are treated with a combination of treatments such as surgery and radiation therapy (Ambroggi et al., 2015).

Nowadays, The patient is commonly given chemotherapy, radiation, immunotherapy, surgery, and hormone treatments. Apart from these therapies, other strategies are also used in combination of these treatment methods. In order to minimize blood loss and promote the patient's well-being, stem cell transplantation is considered as the best cancer treatment option and is carried out following other standard therapies (Saini et al., 2020).

Commonly used methods for cancer treatment can be diversified including surgery, radiation therapy, chemotherapy, immunotherapy, targeted therapy, hormone therapy, stem cell transplants and precision medicine. Surgery is the treatment technique in which lymph nodes are removed to remove cancer from the body and prevent or reduce the spread of the disease. Radiation therapy treats cancer by shrinking tumors and killing cancer cells. However, this method involves exposure to a lot of radiation. Chemotherapy treats cancer by killing cancer cells and shrinking tumors, but it has serious side effects. In this method, various chemicals are used for treatment purposes. Immunotherapy is used to strengthen the immune system. In this treatment, the aim is for the body to defeat the cancer on its own, based on the use of medication or other treatments. Target therapy, on the other hand, strengthens the immune system by providing targeted treatment. It is a form of treatment with drugs that aim to interfere with the mechanism that causes cancer to grow, spread and grow. Hormone therapy mostly treats prostate and breast cancers by stopping and slowing their growth. These types of cancer use hormones to help the cancer grow. Therefore, it is possible to halt and even stop the proliferation of cancer cells by employing hormones as a therapoutic intervention. Damaged tissues and organs lose their capacity to mend themselves as the body's stem cells decline. In stem cell transplantation treatment, stem cells destroyed by very high doses of radiation or chemotherapy in cancer patients are repaired. By performing various genetic

tests, the best treatment for the patient is determined by precision medicine. Precision medicine is an a more innovative method (Saini et al., 2020) (Ambroggi et al., 2015). These treatment methods include many therapeutic drugs such as antibiotics used in chemotherapies, different targeted systems such as nanotechnology to directly treat cancer, microspheres and similar forms of treatment for the same purpose.

Many patients diagnosed with cancer are trying to look for alternative complementary treatment methods such as functional foods and medicinal herbs due to the serious side effects of chemotherapy and radiation therapy on the human body and high mortality rates (Abu-Reidah et al., 2015). Many patients diagnosed with cancer are looking for alternative or complementary treatment methods due to the serious side effects of chemotherapy and radiation therapy (Shah et al., 2013).

2.5.1. Use of plants in cancer treatment

Cancer is among the most resistant diseases in terms of treatment processes and treatment success globally. Since the mechanisms of cancer disease are complex and not fully understood, and cancer cells are very similar to healthy cells, drug treatment cannot provide complete success. Because of this, scientists are still working on finding natural and efficient resources to treat cancer, and this is a worthwhile topic to study. Ethnobotany research has advanced significantly in recent years. (Teklehaymanot and Giday, 2007) (Ağalar et al., 2017).

There is no exact information about the number of plant species used for medicinal purposes in the world, estimates are between 20,000 and 70,000. According to WHO's estimates, 80% of the world's population and 95% of the African population benefit from treatment methods based on medicinal plants. Chemical and pharmacological research has been conducted on only 15% of flowering plants. This rate is quite low when taking into account all of the plant species on Earth (Kendir and Güvenç, 2010). We may conclude that plants are a vast resource that can be used in a variety of medical applications.

Many medications are available to treat various types of cancer. None of these drugs can be said to be completely effective and safe. The toxicity of implanted drugs is the biggest problem in cancer chemotherapy (Prakash et al., 2013). The National Cancer Institute (NCI) studied nearly 35,000 plant species with potentially anti-cancer properties. Among them, approximately 3,000 plant species have shown ongoing anticancer properties (Desai et al.,

2008). New methods are in demand to prevent this disease. An alternative and effective medicine is needed to combat this disease because conventional treatments damage healthy cells. Nonetheless, products made from plants and herbs have shown promise in the management and treatment of cancer. Products from a variety of plants have been demonstrated to be successful in the treatment of cancer. This effect occurs by inhibiting cancer-activating enzymes, stimulating the DNA repair mechanism, inducing antioxidant effect and promoting the production of protective enzymes (Bharadvaja, 2017). Today, most of the research on cancer drugs focuses on plants and natural products derived from plants. The anti-cancer properties of various plants are being brought to light, and many natural products and their analogs have been discovered to be potent anti-cancer agents (Prakash et al., 2013).

According to a study, more than 60% of cancer patients use herbs or vitamins for treatment (Pandey, 2008). Compounds isolated from medicinal plants may not ultimately serve as drugs for therapeutic purposes. Nevertheless, the study has shown that some plant pioneer molecules are useful for the development of possible anticancer drugs (Shah et al., 2013). Various medicinal plant extracts and their secondary metabolites show a strong defense mechanism against cancer. There are many plants with anti-cancer secondary metabolites. *In vitro* studies prove the potential of secondary metabolites in anti-cancer activity, and plant metabolites contribute to their anti-cancer nature in various ways (Bharadvaja, 2017).

It is very important today to identify new, alternative agents for the treatment of fatal NSCLC. The search for alternatives has arisen due to the decrease in the effects of treatment methods and the increase in side effects experienced by the patient due to the drugs used in the treatments. Finding novel, alternative therapy agents is crucial in the current fight against deadly NSCLC. Recently, many researchers have been investigating natural products, especially plant-derived compounds, that can be used instead of or in combination with synthetic compounds used in treatments, due to their potential antitumor properties (zhang et al., 2018).

2.5.2. Natural products and their significance in cancer treatment

The first investigation of plants as anticancer agents in cancer research began in the 1950s with the discovery and development of vinca alkaloids, such as vinblastine and vincristine, and the isolation of cytotoxic podophyllotoxins. of chemical entities, molecular targets and

signaling The US National Cancer Institute (NCI) has also acknowledged the promise of natural products as anticancer medicines, and has played a significant role in the development of novel plant-based anticancer drugs (Cragg and Newman, 2005). Recent research has focused on the identification of critical pathways that these natural chemicals activate or inhibit, in addition to the application of bioactive compounds obtained from natural sources as possible new anti-cancer medicines (Sak and Everaus, 2017). Historically, natural ingredients have been used to treat cancer, especially in traditional Chinese or Indian Ayurvedic medicine.Chemicals with significant pharmacological effects that are present in plants, fungi, marine animals, or microorganisms are referred to as "natural compounds" (Rejhová et al., 2 018).

There are many available anticancer drugs (figure 2.1), such as classical examples of plant-derived molecules, taxanes (Paclitaxel and Docetaxel), vincristine and vinblastine alkaloids, podophyllotoxin derivatives (etoposide and teniposide), and camptothecins such as topotecan (Sak and Everaus, 2017). For example, green tea antioxidant EGCG (epigallocatechin-3-gallate) significantly slows the growth of breast cancer cells. Nature provides a wide variety of compounds to combat tumor growth. Chemical modification of natural molecules can make them even more effective and at the same time reduce their negative effects (Cragg and Newman, 2005). This suggests that many drugs obtained directly from natural sources or derived from the derivation of naturally occurring compounds are clinically useful. Approximately 70% of anticancer drugs approved by the Food and Drug Administration (FDA) in the United States come from natural sources (Amin et al., 2009).

Some side effects on the human body are one of the most important disadvantages of synthetic drug treatments (Tomlinson and Akerele, 2015). Radiotherapy and chemotherapy, traditional methods of cancer treatment, have serious side effects such as neurological, cardiac, renal and pulmonary toxicity (Bharadvaja, 2017). However, using natural remedies rather than synthetic ones to combat cancer may be advantageous when using plants or plant-derived products (Figure 2.2) (Tomlinson and Akerele, 2015). Compounds derived from medicinal plants are more easily tolerated and are not harmful to normal human cells. Therefore, it has various advantages over chemical products (Bharadvaja, 2017).

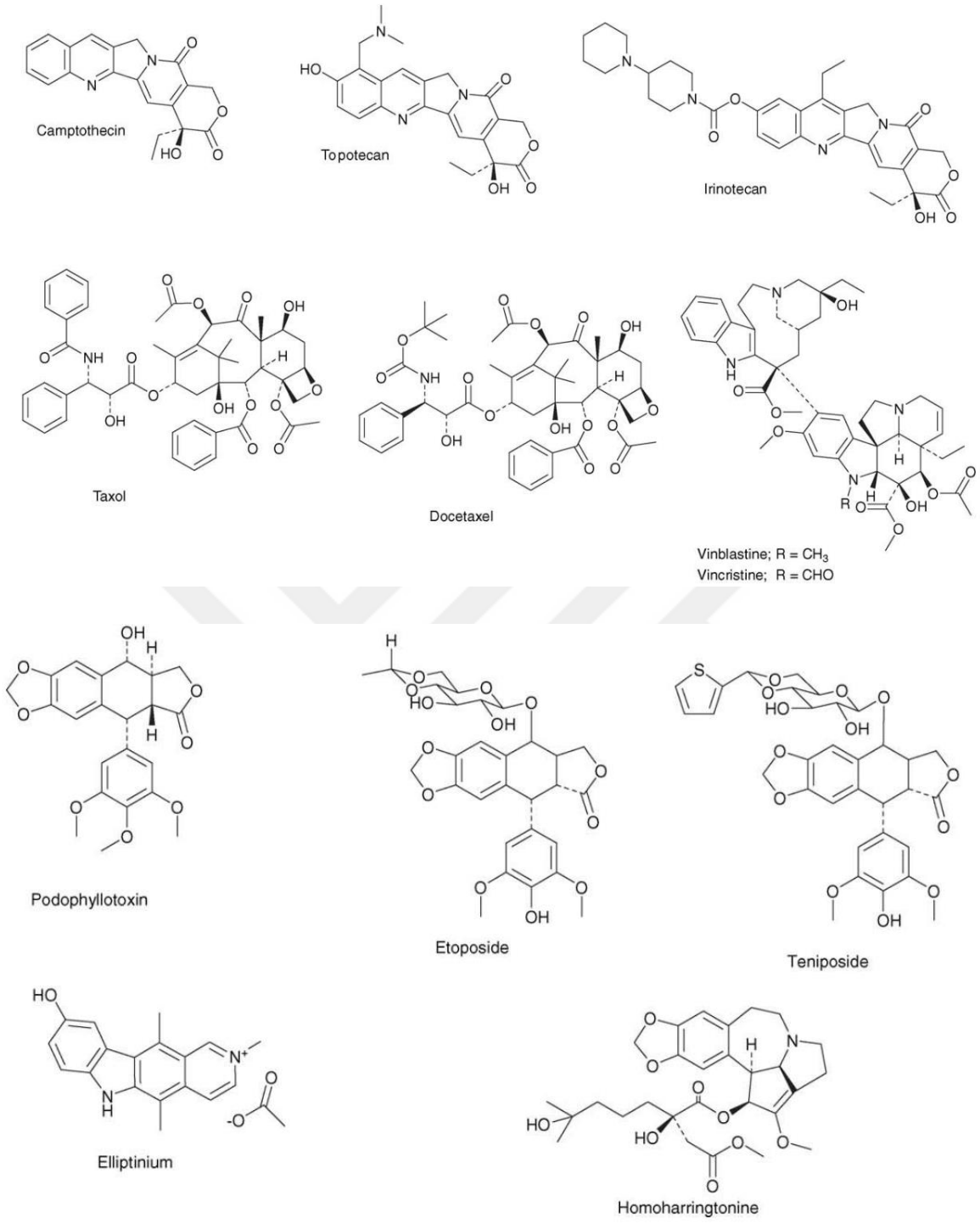


Figure 2.2 Plant-derived anti-cancer agents used in the clinic. Adopted from: (Cragg and Newman, 2005)

In vitro and *in vivo* studies have shown that natural products can increase antitumor efficacy by working with drugs commonly used in tumor treatment. This can occur through various

mechanisms, such as induction of apoptosis and inhibition of cell proliferation, invasion, metastasis and angiogenesis (de Oliveira Júnior et al., 2018).

Table 2.1. Some anti-cancer plants, part used, components and origins. (Prakash et al., 2013)

Botanical name of plant with family name	Part used	Parts used and their main active components	Origin / native place
*Agave americana (*Agavaceae)	Leaf	Steroidal saponin, alkaloid, coumarin, isoflavonoid, hecogenin and vitamins (A, B, C)	Central America
Agropyron repens (Poaceae)	Rhizomes	Rhizome contains essential oil, polysaccharide and mucilage	Europe
Agrimonia pilosa (Rosaceae)	Herb	Agrimonolide, flavonoid, triterpene, tannin and coumarin	China, Japan, Korea, India
Ailanthus altissima (Simaroubaceae)	Bark	Triterpene, tannin, saponin and quercetin-3-glucoside	China, Korea
Akebia quinata (Lardizabalaceae)	Fruit	Flavonoid and saponin	China, Japan, Korea
Alpinia galanga (Zingiberaceae)	Rhizomes	Kaempferide and flavone	Europe
Aristolochia contorta (Aristolochiaceae)	Root and fruit	Lysicamine and oxaaporphine	China, Korea
Aster tataricus (Asteraceae)	Whole plant	Triterpene, monoterpene and epifriedelanol	Japan, Korea
Bryonia dioica	Root	Cucurbitacin and glycoside	Europe
Cannabis sativa (Cannabinaceae)	Leaf	Stereo isomers of cannabitriol	South Africa
Chelidonium jajus var. *asiaticum* (Papaveraceae)	Herb	Alkaloids (sanguinarine, chelerythrine, berberine)	Asia, Europe
*Chimaphila umbellate (*Ericaceae)	Whole plant	Ericolin, arbutin, urson and tannin	Asia, Europe
Coix lachryma jobi (Poaceae)	Seed	Trans-ferulyl stigmasterol	China
Dryopteris crassirhizoma (Polypodiaceae)	Rhizomes	Filicinic and filicic acids, aspidinol and aspidin	China, Japan, Korea
Echinops setifer (Asteraceae)	Whole plant	Echinopsine	Korea
Erythronium americanum (Liliaceae)	Whole plant	Alpha-methylenebutyrolactone	North America
Euonymus alatus (Celastraceae)	Whole plant	Triterpene, euolatin, steroid and sesquiterpene alkaloid	China, Japan, Korea
Eupatorium cannabinum (Asteraceae)	Whole plant	Sesquiterpene, lactone, pyrrolizidine alkaloidand flavonoid	Europe, Asia,
Fragaria vesca (Rosaceae)	Leaf and fruit	Flavonoid, tannin, borneol and ellagic acid Asia, Europe	Asia, Europe
Fritillaria thunbergii (Liliaceae)	Whole plant	Alkaloid and peimine	China, Siberia
Galium aparine (Rubiaceae)	Cleaver	Iridoid, polyphenolic acid, tannin, anthraquinoneand flavonoid	Europe , Africa
Hydrastis canadensis (Ranunculaceae)	Whole plant	Isoquinoline alkaloids (hydrastine, berberine, berberastine, candaline), resin and lactone	Canada, United States
Junchus effuses (Juncaceae)	Whole plant	tridecanone, effusol, juncanol, phenylpropanoid and atocopherol	China, Japan, Korea
Lantana camara (Verbenaceae)	Whole plant	Alkaloids (camerine, isocamerine, micranine,lantanine, lantadene)	Tropical America
Larrea tridentate (Zygophyllaceae)	Whole plant	Resin	Southwestern USA,Mexico
Lonicera japonica (Caprifoliaceae)	Whole plant	Tannins, saponins and carotenoids	China
Olea europrae (Oleaceae)	Leaf and oil	Oleic acid and polyphenol	America

***Panax quinquefolium* (Araliaceae)**	Roots	Ginsenoside, sesquiterpene, limonene vitamins (B_1, B_2, B_{12})	China, Japan, Korea
Phaleria macrocarpa	Fruits	Gallic acid	Indonesia
***Polygonatum multiflorum* (Liliaceae)**	Whole plant	Saponin, flavonoid and vitamin A	Asia, Europe, North America
***Potentilla chinensis* (Rolsaaceae)**	Whole plant	Gallic acid and tannin	China, Japan, Korea
***Pygeum africanum* (Boraginaceae)**	Bark	Phytosterol, triterpene and tannin	Africa
***Pyrus malus* (Rosaceae)**	Bark and fruit	Quercetin, catechin, flavonoid, coumaric and gallic acids, and procyanidin	Britain
***Rhus chinensis* (Anacardiaceae)**	Leaf	Tannin, apigenin and glycoside; seed contains bruceosides (A, B),	China, Japan, Korea
***Rubus idaeus* (Rosaceae)**	Leaf	Flavonoid and tannin; fruit contains vitamins (A, B, C) and ellagic acid	Asia, Europe
***Scilla natalensis* (Hyacinthaceae)**	Bulb	Bulb	South Africa
***Scrophularia nodosa* (Scrophulariaceae)**	Aerial parts	Iridoid, flavonoid and phenolic acid	Europe
***Smilax chinensis* (Liliaceae)**	Rhizomes	Tannin, saponins and flavonoid	China, Japan
***Tabebuia* spp. (Bignoniaceae)**	Bark	Quinine, bioflavonoid and co-enzyme Q	South America
***Thuja occidentalis* (Cupressaceae)**	Whole plant	Flavonoid, tannin, volatile oil and mucilage	Northeastern USA, Europe
***Thymus vulgaris* (Lamiaceae)**	Whole plant	Volatile oil, flavonoid and tannin	South Europe
***Trifolium pratense* (Fabaceae)**	Flower	Glucosides (trifolin, trifolitin, trifolianol), flavonoid	Asia, Europe, Africa, Australia
***Vitex rotundifolia*(Verbenaceae)**	Whole plant	Camphene, pinene and diterpene	China, Japan, Korea

2.6. *Aracea* Family

The *Araceae* family was introduced to the scientific literature by botanist Antoine Laurent de Jussieu at the end of the 1700s, at an altitude of approximately 3000 m. It is a plant family that spreads over a large area from altitude to sea shores and includes 118 genera and 3800 species (Henriquez et al., 2014).

Within its own species, this family contains numerous morphological and ecological modifications. Among the known monocot groups, it is the most varied (Zhao et al., 2023). Tropical regions are home to 95 % of the species and 90 % of the taxa in the *Araceae* family. In terms of habitat and vegetative characteristics, species of this family can be found in a wide variety of forms. Although most of the plant species belonging to the *Araceae* family are used as indoor ornamental plants, some species have high medicinal value, their compounds have been researched and are consumed by the public (Çeçen et al., 2019).

2.7. *Arum* Genus

The genus *Arum*, which belongs to the *Araceae* family, includes 28 species and covers a wide area from Europe to Asia. Such plants are perennial, herbaceous, tuberous, and poisonous. They have colorful spatas with long-stemmed, triangular-shaped leaves. In our country, this plant naturally lives in moist or swampy areas, in some forests, streamside slopes, bushy areas, and among the rocks and stones (Değer, 2022). In practically every part of our nation, local names are employed for a variety of reasons. Taxa belonging to the genus *Arum*, the most common of which is called "snake pillow", are "snake knife, elephant's ear, calf's foot, gavur beet, bitter beet, henna of Ahu, Hemorrhoid grass, bitter onion, livik, nivik, calf grass, pork lettuce, pork cabbage, kari, nevik and snake bead" it is also called (Baytop, 2015).

Consuming *Arum* species unconsciously may cause some health problems. For example, consuming fresh leaves and tubers can result in diarrhea, nausea, vomiting, and even arrhythmias. Fresh leaves and tubers also contain mucilage, saponin, gum, starch and conisin alkaloid. Tubers are used externally for rheumatism. In addition, after drying, the tuber parts are used as an expectorant and breast emollient in dermatological disorders, and as a mixture of granulated tuber and honey in stomach ulcers. Since it is also included in animal feed, it increases milk yield in lactating animals (Stankovi, 2011).

Arum species are also used in traditional treatment methods among the public. Numerous medical conditions, including asthma, bronchitis, intestinal laziness, bladder and urinary tract disorders, malaria, hemorrhoids, shortness of breath, and skin infections, are treated with *Arum* species. They also use the juice obtained from the seeds of this plant to treat ear infections (Değer, 2022). It is also claimed that the extract created by boiling the leaves and extracting their water has anticancer properties (Uçan Türkmen et al., 2019).

2.8. *Arum Dioscoridis* L. Plant

The public has been using the plant species *Arum dioscoridis* L. for millennia. Even animals avoid eating the poisonous monocotyledon and angiosperm *Arum dioscoridis* L. There are 3 varieties of this plant*:* *Arum dioscoridis* Sm. var. dioscoridis, *Arum dioscoridis* Sm. var. luschanii R.Mill and *Arum dioscoridis* Sm. var. spectabile (Schott) Engler (Uçan Türkmen et al., 2019).

This plant species is a tuberous plant standing vertically on the soil surface. Tuber sizes range from 3-5 x 3-6 cm. Leaf stalks are in the range of 10-55 cm x 3-9 mm. The flowers smell intensely of animal manure and carrion. The flowers are usually shorter than the leaves and rarely the same size, ranging from 7-45 cm x 3-10 mm. While the spata is 12-41 cm long, the spata tube is 2.5-6 x 1.5-3.5 cm and its inner surface is greenish white and sometimes purple towards the tip. The female flower area is 7-18 mm tall; The ovaries are light yellow to light green in color. The male flower part is 4-8 mm long and the anthers are ivory-colored (Değer, 2022). The size of a plant that has completed its development and bloomed is between 30-50 cm. Its leaves are arrow-shaped, long-stemmed and dark green in color (Figure 2.2). Its flowers, which bloom in spring, are observed in the shape of a cone, and the female flowers are at the bottom and the male flowers are at the top. With pea-sized orange seeds grouped together and resembling a corn cob in structure, it develops near the end of summer. In the Eastern Mediterranean region, the leaves of the *Arum dioscoridis* L. plant are also consumed as food in household kitchens. The leaves of this plant have a bitter taste. Therefore, to consume it as food, remove this bitter taste, the leaves are boiled, filtered and then prepared for cooking (UĞUZLAR, 2009). In Osmaniye, Adana and Kahramanmaraş regions, the leaves and petioles of this plant are mixed with yogurt to make "tirşik", a local dish. Due to its toxicity, it is not eaten in some areas (Uçan Türkmen et al., 2019). Despite the paucity of research on *A. dioscoridis*, Asians, Europeans, and Turks are familiar with the *Arum* taxonomic family (Yabalak, 2018).

Although the healing properties of the *Arum dioscoridis* L. plant have not been proven by clinical research, research shows that this plant is still widely used in traditional folk medicine, especially among the public (Emre et al., 2021). It is used as a herbal remedy in Anatolia to treat a variety of conditions, including mumps, snakebite, bladder and urinary tract disorders, malaria, expectoration, intestinal lethargy, shortness of breath, asthma, bronchitis, chest softness, and hemorrhoids. The regions where our country grows, especially Adana, are the Aegean, Mediterranean, Marmara, Black Sea and Southern Anatolia, and Cyprus regions. In these regions, it grows wild in cemeteries, fields and gardens, stream beds, roadsides, and empty lands (Değer, 2022).

A.

B.

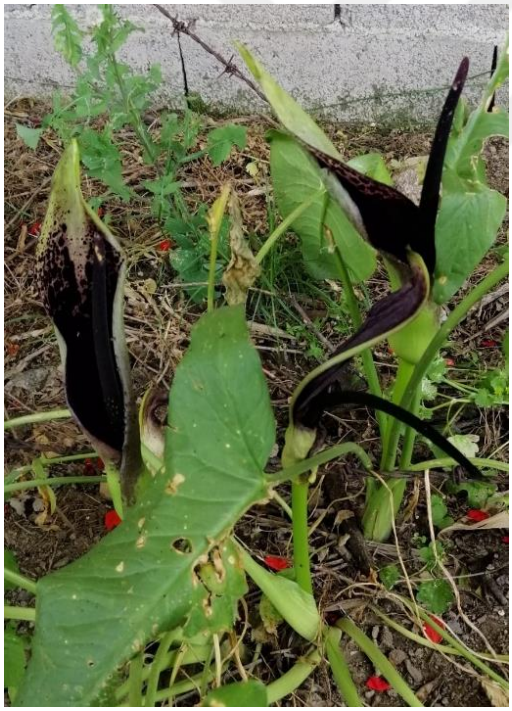


Figure 2.3 *Arum dioscoridis* L. plant leaves, seeds and flowers. A) is part of seeds and B) flowers and leaves.

2.8.1. The role of *Arum dioscoridis* L. in cancer treatment.

Arum subspecies can produce extra heat energy by increasing the body's metabolic rate or shivering (Azab, 2017).

In a study, the extract contents of *Arum dioscoridis* L., which grows naturally in our country, were examined by the GS-MS method, and 16 different compounds of pharmaceutical and medical importance detected in the MeOH ic extract were obtained. *Arum dioscoridis* L. MeOH ic plant extract, which has demonstrated antioxidant properties and contains high amounts of phenolic compounds, appears to be a plant extract worth using for cancer research (Yabalak, 2018).

Vitexin was found to be 4.1 mM in the extraction using HPLC-DAD in a research that involved drying the Arum dioscoridis L. plant and performing an ethanol extraction. Tozasertib (cancer drug) and Extract were applied to CFPAC-1 (pancreatic cancer) cells for concomitant effect, Annexin V apoptosis test and DNA cell cycle analysis were performed. Drug and extraction led cancer cells to apoptosis at a high rate. CASP-3 gene expressions are observed to increase by 2 times in both. Furthermore, DNA cell cycle analysis in CFPAC-1 cells revealed a very low S phase level in this treatment combination. It was discovered that cells exposed to Tozasertib were arrested in the G2 phase, whereas cells exposed to the extract were arrested in the G1 phase. With wound dehiscence experiments, it was observed that migration stopped in all groups containing extract. It is observed that there was a 40% decrease in the levels of VEGF gene expression, which plays a role in angiogenesis, in the groups containing the extract (Ozturk, 2017). *Arum dioscoridis* L. plant extract has been demonstrated to have anti-migratory properties. It is one of the medicinal plants that has been shown to have anticancer properties in a number of research.

2.8.2. Chemical composition of *Arum dioscoridis* L.

The toxicity of these plants is one of the most important characteristics of *Arum. Arum* subspecies has toxicity from several single compounds or families of compounds. (Azab, 2017).

The plant has been the subject of various studies (Table 2.1) because it is promising in terms of its flavonoid content. In these studies, the highest extraction efficiency was determined in extracts using highly polarized distilled water (27.38%) and MeOH (24.21%) solvents.

According to Uçan Türkmen et al. (2019), the extraction efficiency percentages for low polarity acetone and diethyl ether solvents were 9.5% and 8.60%, respectively. Six flavonoids, one coumarin glycoside and two phenolic acids were detected in the ethanol extract of *Arum dioscoridis* L.. Identified components were (in mg mL -1) isoorientin (88.31), vitexin (36.27), luteolin (4.97), apigenin (0.35), quercetin-3-O-β-glucoside (0.63), quercetin (0.15), esculin (0.30), ferulic (4.09) and caffeic acids (2.72) respectively (Afifi et al., 2016).

Table 2.2. Chemical compositions of different plant parts in *Arum dioscoridis* L.

GROUPS	NAME OF CHEMİCALS	PARTS OF PLANT	ANALYSİS METHOD	METHOD OF EXTRACTİON	AMOUNT OF CHEMİCALS	REFERANCES
OTHER TYPE ACİDS	Hexadecanoic acid 2-Hydroxy Metil Ester	Seed	HPLC-MS Analysis	Acetone	4,50 %	(UĞUZLAR, 2009)
		Seed	GC	Acetone	5.50±0.75 % [1]	(Uguzlar, Maltas, and Yildiz, 2011)
	Ethanedioic acid Dimethyl Ester	Seed	HPLC-MS Analysis	Hexane	3,32%	(UĞUZLAR, 2009)
		Seed	GC	Acetone	4.72±0.19 % [1]	(Uguzlar et al., 2011)
	Levulinic acid Metil Ester	Seed	GC	MeOH	19.58±1.05 % [1]	(Uguzlar et al., 2011)
				Acetone	6.65±0.39 % [1]	
		Seed	HPLC-MS Analysis	MeOH	19,58%	(UĞUZLAR, 2009)
		Seed	HPLC-MS Analysis	Acetone	4,65 %	
	Hepta 2,4 – Dieonic acid Metil Ester	Seed	HPLC-MS Analysis	MeOH	16,16 %	(UĞUZLAR, 2009)
		Seed	HPLC-MS	Acetone	1 %	

			Analysis			
		Seed	GC	MeOH	16.16±2.12 % [1]	(Uguzlar et al., 2011)
				Acetone	2.00±0.55 % [1]	
	Butanedioic acid Methyl Dimethyl Ester	Seed	HPLC-MS Analysis	MeOH	2,02 %	(UĞUZLAR, 2009)
		Seed	HPLC-MS Analysis	Acetone	0,78 %	
		Seed	GC	MeOH	2.02±1.17 % [1]	(Uguzlar et al., 2011)
				Acetone	1.78±0.21 % [1]	
	Propanedioic acid Dimethyl Ester	Seed	HPLC-MS Analysis	Hexane	0,40 %	(UĞUZLAR, 2009)
FATTY ACİDS	Capric acid	Seed	GC-MS	Hexane	0,4 %	(UĞUZLAR, 2009)
	Palmitik acid	Seed	GC-MS	MeOH	16,11 %	(UĞUZLAR, 2009)
				Acetone	23,60 %	
				Hexane	24,53 %	
	Steraric acid	Seed	GC-MS	MeOH	1,70 %	(UĞUZLAR, 2009)
				Acetone	3,12 %	

FATTY ACİDS				Hexane	3,25 %	
	Palmit oleic acid	Seed	GC-MS	MeOH	3,31 %	(UĞUZLAR, 2009)
				Acetone	4,15 %	
				Hexane	3,43 %	
	Oleic acid	Seed	GC-MS	MeOH	8,18 %	(UĞUZLAR, 2009)
				Acetone	12,39 %	
				Hexane	13,06 %	
	Linoleic acid	Seed	GC-MS	MeOH	18,03 %	(UĞUZLAR, 2009)
				Acetone	21,90 %	
				Hexane	27,02 %	
	Linolenic acid	Seed	GC-MS	MeOH	3,12 %	(UĞUZLAR, 2009)

				Acetone	4,30 %	
				Hexane	6,44 %	
	Lauric acid	Seed	GC-MS	Acetone	0,46 %	(UĞUZLAR, 2009)
	Miristic acid	Seed	GC-MS	Acetone	0,90 %	(UĞUZLAR, 2009)
				Hexane	0,78 %	
PHENOLIC PLANT ACIDS	Cafeic acid	Seed	HPLC	Hexane	5,6 (µg/ml)	(UĞUZLAR, 2009)
		Leaves and flowers	HPLC-MS	Ethanol	2,72 (mg mL−1)	(Afifi, Kasabri, Litescu, and Abaza, 2016)
	Ferulic acid	Seed	HPLC	MeOH	325,5 (µg/ml)	(UĞUZLAR, 2009)
				Acetone	255,9 (µg/ml)	
		Leaves and flowers	HPLC-MS	Ethanol	4,09 (mg mL−1)	(Afifi et al., 2016)
		Seed	HPLC	MeOH	325.5 ± 2.5 (µg/g) [1]	(Uguzlar, Maltas, and Yildiz, 2012)

				Acetone	255.9 ± 1.9(µg/g) [1]	
	P-Coumaric acid	Seed	HPLC	MeOH	5,4 (µg/ml)	(UĞUZLAR, 2009)
				Acetone	5,6 (µg/ml	
				MeOH	5.4 ± 0.8 (µg/g) [1]	(Uguzlar et al., 2012)
				Acetone	5.6 ± 0.4 (µg/g) [1]	
FLAVONOIDS	Vitexin	Seed	HPLC	MeOH	1125,0 (µg/ml)	(UĞUZLAR, 2009)
				Acetone	1411,6 (µg/ml)	
				Hexane	23,4 (µg/ml)	
		Leaves and flowers	HPLC-MS	Ethanol	36,27 (mg mL−1)	(Afifi et al., 2016)
		Seed	HPLC	MeOH	1,125.0 ±13.4 (µg/g) [1]	(Uguzlar et al., 2012)
				Hexane	23.4 ± 2.4 (µg/g) [1]	

FLAVONOIDS	Naringin	Seed	HPLC	MeOH	98.4 ± 5.5 (µg/g) [1]	(Uguzlar et al., 2012)
				Acetone	19.4 ± 3.2 (µg/g) [1]	
		Seed	HPLC	MeOH	98,4 (µg/ml)	(UĞUZLAR, 2009)
				Acetone	98,4 (µg/ml)	
	Eriodictyol	Seed	HPLC	MeOH	43,7 (µg/ml)	(UĞUZLAR, 2009)
				Acetone	55,0 (µg/ml)	
				Hexane	5,2 (µg/ml)	
				MeOH	43.7 ± 3.1 (µg/g) [1]	(Uguzlar et al., 2012)
				Acetone	25.0 ± 4.2 (µg/g) [1]	
				Hexane	5.2± 0.7 (µg/g) [1]	
	Isoorientin	Leaves and flowers	HPLC-MS	Ethanol	88,31 (mg mL−1)	(Afifi et al., 2016)

	Luteolin	Leaves and flowers	HPLC-MS	Ethanol	4,97 (mg mL−1)	(Afifi et al., 2016)
	Apigenin	Leaves and flowers	HPLC-MS	Ethanol	0,35 (mg mL−1)	(Afifi et al., 2016)
	Quercetin 3-O-β-glucoside	Leaves and flowers	HPLC-MS	Ethanol	0,63 (mg mL−1)	(Afifi et al., 2016)
	Quercetin	Leaves and flowers	HPLC-MS	Ethanol	0,15(mg mL−1)	(Afifi et al., 2016)
COUMARIN GLYCOSIDE	Esculin	Leaves and flowers	HPLC-MS	Ethanol	0,30 (mg mL−1)	(Afifi et al., 2016)

2.8.3. Potential anticancer chemicals from *Arum dioscoridis* L.

When the extract of *Arum dioscoridis* L. in different solutions was examined, it was shown that the content of flavonoid substances such as isoorientin and vitexin was quite high (Bozbey et al., 2015).

The anticancer properties of these flavonoid ingredients have been independently proven in various studies. *Arum dioscoridis* L. plant, which contains all of these substances, is promising in terms of its anticancer properties (Bozbey et al., 2015).

2.8.3.1. Vitexin

Vitexin is a flavonoid and lignan compound found naturally in various plant sources. Anti-cancer effects of vitexin in vitrowere observed in breast adenocarcinoma (MCF7), bladder carcinoma (T24), cervical carcinoma (HeLa), colorectal carcinoma (HCT116andLoVo), malignant melanoma (A375, Sk-Mel-5, and Sk-Mel-28). has been shown in glioblastoma

(LN18) and lung cancer (A549). Vitexin's anti-cancer effects in vivo have been demonstrated in hepatocellular, colorectal, lung carcinoma and melanoma in mice. An anti-cancer effect has been observed in mice by reducing tumor growth and size in vivo (Peng et al., 2021). Vitexin inhibits cell viability and AktandmTOR signaling in human glioblastoma cells. It also induces cell cycle arrest and apoptosis in the G2/M phase in glioblastoma cells (Zhang et al., 2018). Vitexin inactivates PI3K/Akt/mTOR signaling in lung cancer (A549). Vitexin reduces the viability of A549 cells with almost no toxic effect on human bronchial epithelial cells. Additionally, vitexin promotes A549 cells to undergo higher rate of apoptosis while lowering the Bcl-2/Bax ratio (X. Liu et al., 2019)..

2.8.3.2. Isoorientin

A naturally occurring C-glucosyl flavone, isoorientin has a wide range of effects due to its several pharmacological properties (Ziqubu et al., 2020). Isoorientin; Studies have shown that it has antitumoral activity on lung, hepatocellular carcinoma, oral squamous cell carcinoma (OSSC), pancreas and colorectal adenocarcinoma (Lin et al., 2016; S. C. Liu et al., 2021; Y. Xu and Qian, 2014). In a study conducted on the A549 lung cancer cell line, it was shown that Bax increased the level of Cle-caspase-3 and decreased the level of Bcl-2, and also induced apoptosis through the ROS-related MAPK/STAT3/NF-kappaB signaling pathway (W. T. Xu et al., 2020). In the study using isoorientin from Gypsophila elegans, it was shown that it caused a remarkable apoptotic death in HepG2 hepatocellular carcinoma cells by inhibiting the G1 phase through the regulation of cell cycle-related genes such as cyclin D, cyclin E and, CDK2. It was also noted that it significantly increased ROS formation and the Bax/Bcl-2 ratio in this cell line (Lin et al., 2016).

In the study conducted on OSCC, it was shown that isoorientin could prevent cancer metastasis and recurrence with its inhibitory effect on WNT/beta-catenin/STAT3 (S. C. Liu et al., 2021). In a study conducted on pancreatic cancer cells, isoorientin was shown to induce apoptosis, reduce invasion and reduce VEGF secretion by inhibiting BAX expression and increasing the expression of E-cadherin and Bcl-2 through the AMPK signaling pathway (Ye et al., 2016). When the effect of isoorientin was investigated in HT29 human colorectal adenocarcinoma cells, it was found that the expressions of cyclin D1, cyclin-dependent kinase 6, Bax, Bcl-2, checkpoint kinase 1-2, ERCC1 decreased. An increase in p53, p51, caspase 3-8-9 gene expressions, and activation of the ATR pathway were observed (Gundogdu et al., 2018).

2.9. Aim of the Study

The purpose of this study is to extract natural compounds from seeds of *Arum dioscoridis* L. plants, which are widely found in the Mediterranean area, and to examine their anti-cancer effects primarily on lung cancer cells *in vitro*. For this purpose, several cell biological methods were employed. Particular cancer cell properties such as cell viability, migration and colony formation abilities were tested in the presence of the plant extract. Furthermore, the effect of the extract on cancer cell cycle progression and its capacity to trigger apoptosis were also determined.

3. MATERIALS AND METHODS

3.1. Materials

Roswell Park Memorial Institute-1640 medium (RPMI-1640, Biowest, Nuaille, France),fetal bovine serum (FBS, Biowest, Nuaille, France), phosphate-buffered saline (PBS, Biowest, Nuaille, France), 0.25 % Trypsin- Ethylenediaminetetraacetic acid (Trypsin-EDTA, Gibco, MA, USA), penicillin-streptomycin (PS, Gibco, MA, USA), Dimethylsulphoxide (DMSO, Merck, NJ, USA), 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyltetrazolium bromide (MTT) solution, 1 % aqueous crystal violet solution, powder agar, agarose (Sigma Aldrich, MO, USA) were purchased from commercial resources.

3.2. Methods

3.2.1. *Arum dioscoridis* L. plant materials

Seeds of *Arum dioscoridis* L. were collected from the banks of Osmaniye/Sumbas/Reşadiye streams at the end of August (figure 3.1), which is the seed formation period of the plant. After the seeds were collected, they were stored in a refrigerator bag at -80°C until the date of use.

A.

B.

Figure 3.1 Seeds of the locally grown *Arum dioscoridis* L. plants used for extraction. A) seeds of the plant and B) morphological image of the plant.

3.2.2. Extraction and fractionation of *Arum dioscoridis* L. plant samples

To extract phenolic compounds, water and MeOH , which are highly polar solvents, were found to be suitable solvents for *Aru m dioscoridis* L. plant seeds (Stankovi, 2011).

In the preparation phase of the extraction process, *Arum dioscoridis* L. seeds were dried. Dried seeds were ground and turned into small pieces. The ground seeds were passed through a metal strainer to separate smaller pieces. A mixture of 15 g of ground seeds and 150 ml of total solvent (75 ml dH_2O + 75 ml MeOH) was prepared in a screw cap shot bottle. The mixture was left in an ultrasonic water bath at 37°C, shaking by hand at ≃approximately 5 minute intervals, for a total of 45 minutes. A magnet was put inside the screw cap shot bottle and it was left to mix at room temperature on a magnetic stirrer at medium stirring level for 24 hours. The mixture was filtered using a Buchner funnel. MeOH in the mixture liquid was evaporated by rotating it in a rotary evaporator for ≈ 25 minutes. The mixture removed from MeOH was poured into sterile petri dishes and frozen at -80°C for 72 hours. It was dried in a freeze-dry (lyophilizer) at 0.5 vacuum setting, at -86°C for 48 hours (Figure 3.2). The liquid-evaporated samples were scraped from the petri dishes and weighed. DH_2O filtered with a

0.22 µm filter tip was used as the solvent. It was prepared as a stock solution at a concentration of 5 mg/ml in microfuge tubes. As a result, the obtained HMPEs (1:1) were stored at +4 °C for further applications.

a) 1st hour

b) 48th hours

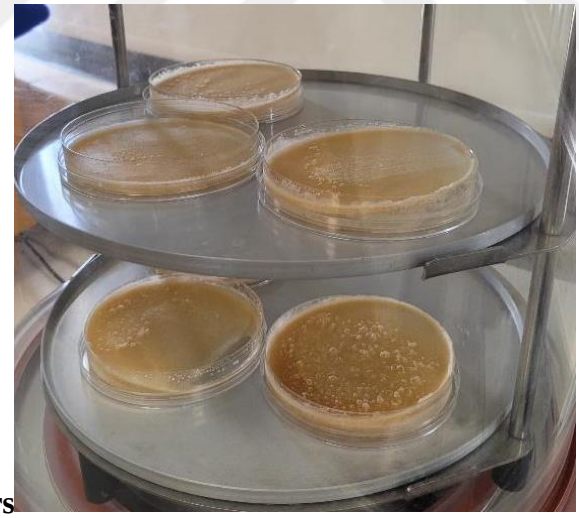


Figure 3.2 This images of samples dried in freezedrier at different times are (a) is 1st hour; (b) is 48th hours.

3.2.3. Cell lines and culture conditions

To examine the anti cancer effects of hydromethanolic plant extracts, HUH-7, MCF-7, BEAS-2B and A549 cell lines were used. The cell lines used in the study was a gift from Prof.

Dr. Ece Şimşek of Antalya Akdeniz University. The characteristics of A549, HUH-7, MCF-7, BEAS-2B and A549 non-small cell lung cancer cells are shown in Table 3.1.

Table 3.1 Characteristic features of cell lines used in experiments.

	A549 (CRM-CCL-185 ™)	**BEAS-2B (CRL-3588 ™)**	**MCF-7 (HTB-22 ™)**	**HUH-7 Cell line**
Organism	*Homo sapiens* , human	*Homo sapiens* , human	*Homo sapiens* , human	*Homo sapiens* , human
Tissue	Lung	Lung; Bronchus	Breast; Mammary gland	Liver
Morphology	Epithelial-like	Epithelial	Epithelial	Epithelial-like
Culture Properties	Adherent	Adherent	Adherent and/or suspension	Adherent
Disease	Carcinoma	Normal	Adenocarcinoma	Hepatocellular carcinoma
Age	58 years	21 years	69 years	57 years
Gender / Ethnicity	Male	Male	Female / White	Male, Japanese

The cells were cultured in RPMI-1640 growth medium prepared by adding 10% fetal bovine serum and 1% penicillin-streptomycin. The cells were cultured in a 37°C incubator containing 5% CO_2 and the cells were checked every day. The subculture protocol was applied when the cells reached 80% density. Subculturing is accomplished by removing the medium, washing the cells with phosphate-buffered saline (PBS), and adding trypsin-EDTA to detach the cells from the flask. The cells removed from the flask are transferred to a new culture medium at the desired cell density or transferred to the flask or petri dish to be used for the respective experiment.

3.2.4. Effects of hydromethanolic plant extracts on A549, MCF-7, BEAS-2B, HUH-7 cell viability

Cell viability of A549, MCF-7, Beas-2B, and HUH-7 cell lines was evaluated using MTT, a colorimetric-based assay to determine the effect of hydromethanolic extract of *Arum dioscoridis* L. seeds. The effect of hydromethanolic extract was evaluated by measuring the conversion of yellow 3-(4,5-dimethylthiazol2-yl)-2,5-diphenyltetrazolium bromide salt into purple formazan crystals via mitochondrial dehydrogenase activity. The color intensity of the obtained formazan crystals was measured in the reference range of 570nm-690nm using the SPECTROstar Nano microplate reader. A549, MCF-7, Beas-2B, and HUH-7 cells were seeded in 96-well plates for MTT analysis at a density of 1x104 cells per well in 100 µL volume. The plates were then incubated at 37 °C for 24 h in an environment containing 5%

CO_2. After 24 h, the existing cell growth medium in the 96-well plate was vacuumed. The treatment medium was added to the wells. Hydromethanolic extraction of *Arum dioscoridis* L. on A549, MCF-7, Beas-2B, and HUH-7 cells was used as the treatment medium. The treatment medium was prepared as stock at a concentration of 50 mg/ml. The initial concentration in the cells was determined as 10 mg/ml as the treatment medium and the concentration (1/2) was diluted by half by serial dilution. The cell medium in which the cells were treated in the 96-well plate was, respectively; medium only, medium with dH_2O in equal volume to treatment, 10 mg/ml treatment medium, 5 mg/ml, 2.5 mg/ml , 1.25 mg/ml , 0.625 mg/ml , 0.31 mg/ml , 0.156 mg/ml , 0,0781 mg/ml extract containing media.

A549, MCF-7, Beas-2B, and HUH-7 cells were incubated with different concentrations of process medium for 24 hours, 48 hours and 72 hours before the experiment. After 24-48-72 hours, the medium was vacuumed over the cells and 100 µl of medium + MTT was added to the wells at a ratio of 1:10. Then, MTT + medium was added to the cells and they were incubated for approximately 3 hours. After incubation, the MTT in each well was vacuumed. Then, 100 µl of DMSO was added to each well. The 96-well plates were shaken with a plate shaker for 15 minutes and centrifuged at 570 nm-690 nm and read on a spectrophotometer.

3.2.5. Wound healing assay

A wound healing assay was conducted to evaluate the impact of the hydromethanolic extract on the migration capability of A549 cells.

In the first stage of the experiment, cells were seeded in a 6-well plate at $2.5x10^5$ cells in each plate and incubated for 48 hours. After 48 hours of incubation, the cells were examined under a microscope. The cell wound healing experiment was performed when the cells covered 80% of the cell culture plate. The 6-well plate was placed in a laminar flow cabinet and the old medium was vacuumed. To create a wound in each well, a ruler was positioned to line up with the marks on both sides of the 6-well plate and intersect the middle. The ruler was then fixed in place and a line was meticulously drawn by gently pressing the 200 µL micropipette towards the ruler. To remove cell debris resulting from the scratch, 1 mL of PBS was added to each well and washed. *Arum dioscoridis* L. hydromethanolic seed extraction on A549 cells was used as the treatment medium. Treatment medium was prepared as stock at a concentration of 50 mg/ml.The initial concentration in the cells as the treatment medium was determined as 10 mg/ml, and the concentration was diluted by half by (1/2) serial dilution.

The cell medium in which the cells were treated on the 6 well plate are respectively; medium + dH_2O, 10 mg/ml, 5 mg/ml , 2.5 mg/ml , 1.25 mg/ml , and 0.625 mg/ml extract containing treatment media. Each well of the cells given treatment medium was photographed under the microscope from the same areas at intervals of 0, 24, 48, 72 hours. Cell images were photographed using a Leica EZ50 dissecting microscope, while identification of wound healing was conducted using Image J software.

3.2.6. Soft agar colony formation assay

The soft agar colony formation assay was used to evaluate the effect of MeOH extract of the *Arum dioscoridis* L. plant seeds on the colony-forming ability of A549 cell lines. Initially, a 2.5% agar solution was prepared and heated in the microwave for 3 min until melted. Then, it was kept in a 56°C water bath for a while to prevent it from solidifying. It was mixed with a pre-warmed RPMI-1640 growth medium. The mixture was used to coat 6-well plates.

For the top agarose layer, the agarose was heated in the microwave for 3 minutes until melted. Then, it was kept in a 40°C water bath for a while to prevent solidification. A mixture of $2x10^4$ cells/ml cells, RPMI-1640 growth medium, and 1.5% agarose in PBS was prepared. This mixture was carefully transferred to the wells without damaging the agar base. After a 24-hour incubation period, cells were fed every after 3 days with RPMI-1640 growth medium containing MeOH extract of *Arum dioscoridis* L. seeds. For this, cells suspended in a medium containing agar were treated with hydromethanolic seed extract at concentrations of 10 mg/ml, 5 mg/ml, 2.5 mg/ml, 1.25 mg/ml, 0.625 mg/ml. The established colonies were after three weeks stained with 0.01% crystal violet dye and photographed using a Leica EZ4 HD dissecting microscope. Identification of colonies was done using Image J software.

3.2.7. Apoptosis assay with AnnexinV-PI-DAPI staining

Apoptosis analysis was performed using DAPI dye (4′,6-diamidino-2-phenylindole), propidium iodide (PI), and Annexin V to examine apoptosis in the A549 cell line of hydromethanolic plant seed extract obtained from *Arum dioscoridis* L.

First, A549 cells were cultured at $1x10^6$cells/24well. After 24 hours of incubation, the serum-containing medium of the cells was removed and incubated with serum-free medium for 24 hours. After 24 hours, the serum-free medium was removed. Hydromethanolic extract was added to the wells at concentrations of control (medium+dH_2O), 10mg/ml, 5mg/ml,

2.5mg/ml, 1.25mg/ml, 0.625mg/ml, respectively, and incubated for 48 hours. After 48 hours of incubation, after washing with PBS for each concentration for analysis, they were stained with DAPI, Annexin V, PI and imaged. Images were taken using a fluorescence microscope.

3.2.8. Cell cycle analysis with flow cytometry

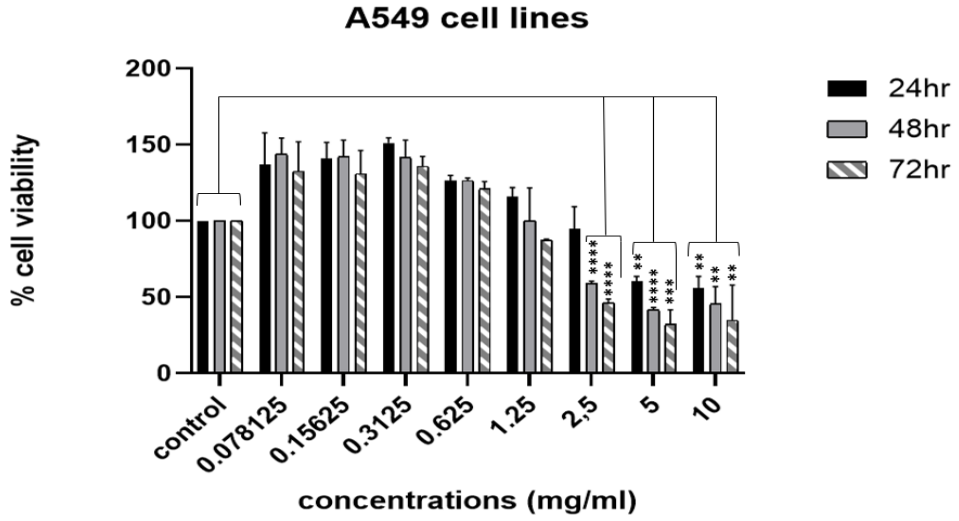
Cell cycle analysis was performed using RNAse-A and propidium iodide (PI) to examine the effects of hydromethanolic plant extract obtained from *Arum dioscoridis* L. plant on cell cycle progressions in A549 cell lines. For this analysis, firstly, cells were seeded as $1x10^6$ cells/petri dish and treated with 2.5 mg/mL of hydromethanolic plant extract for 48 hours. The cells were washed with cold PBS and fixed with 70% ethanol for 3 hours. After the supernatant was removed, RNAase-A and PI were added, and the cells were incubated in the dark for 40 minutes. After centrifugation, the supernatant was removed. Finally, each cell suspension sample was placed in 1.5 ml centrifuge tubes and analyzed by Novocyte (Agilent) flow cytometry device and cell cycle analysis program.

4. RESULTS

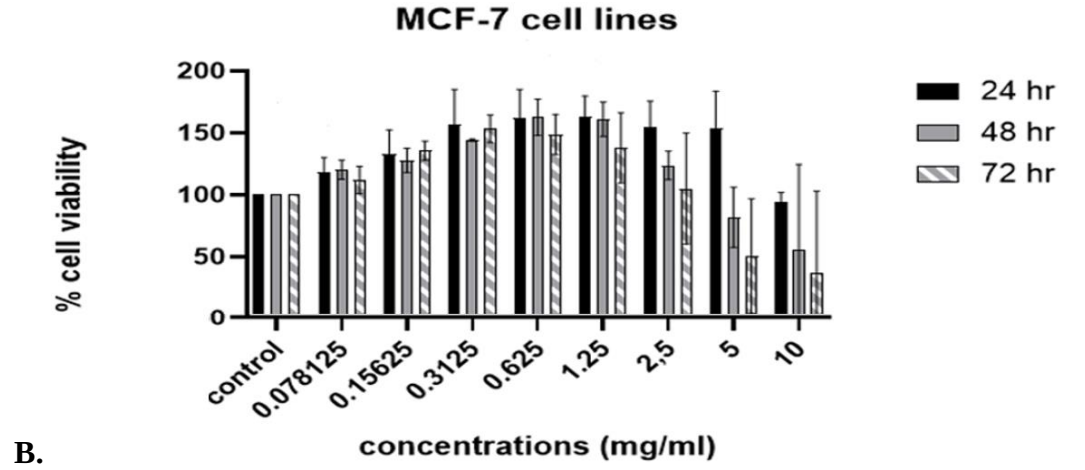
4.1. Effect of Hydromethanolic Plant Extracts on A549, HUH-7, MCF-7, and BEAS-2B Cell Viability

The effects of the prepared with hydromethanolic plant extract solvent obtained from *Arum dioscoridis* L. plant seeds on cell viability in the A549, HUH-7, Beas-2b, MCF-7 cell lines were determined by MTT cell viability test. As a result of the MTT test, spectrophotometer values were taken. These values were tabulated in Excell and Graphpad prism programs and averages, standard deviations, t test were calculated. According to the test results, the graphics in Figure 4.1 were created.

As a result of the test, it was determined that the hydromethanolic plant extract had an adverse effect on cell viability ~~on~~ in the cells exposed to the extracts for 24, 48, 72 hours. For A549 cell lines IC_{50} value in cells incubated for 24 hours was 5,494mg/ml; IC_{50} value in cells incubated for 48 hours was 3.759mg/ml; The IC_{50} value in cells incubated for 72 hours was determined as 2.837mg/ml. For MCF-7 cell lines IC_{50} value in cells incubated for 24 hours was 11.17mg/ml; IC_{50} value in cells incubated for 48 hours was 10.51mg/ml; The IC_{50} value in cells incubated for 72 hours was determined as 6.579mg/ml. For HUH-7 cell lines IC_{50} value in cells incubated for 24 hours was 71.49mg/ml; IC_{50} value in cells incubated for 48 hours was 2.785mg/ml; The IC_{50} value in cells incubated for 72 hours was determined as 1.908 mg/ml. For Beas-2b cell lines IC_{50} value in cells incubated for 24 hours was 7.959 mg/ml; IC_{50} value in cells incubated for 48 hours was 3.063 mg/ml; The IC_{50} value in cells incubated for 72 hours was determined as 1.489 mg/ml.



A.



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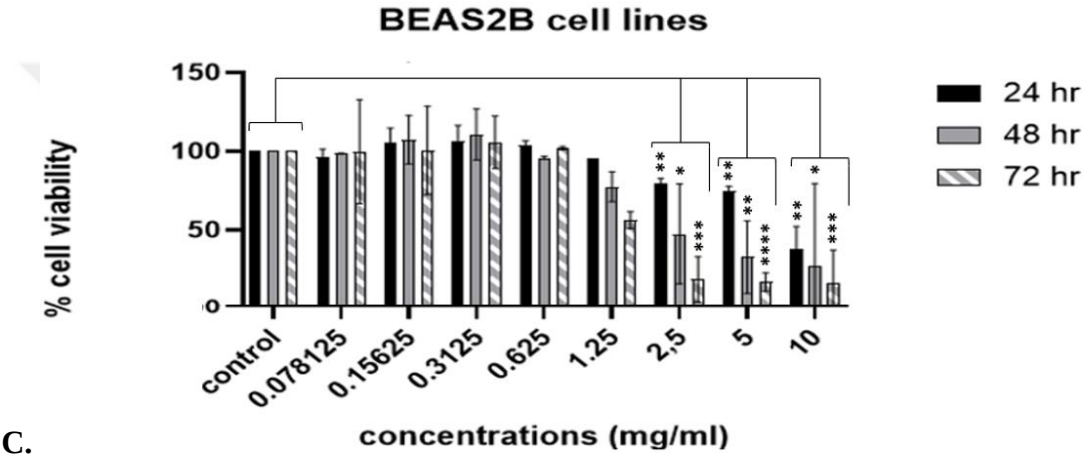

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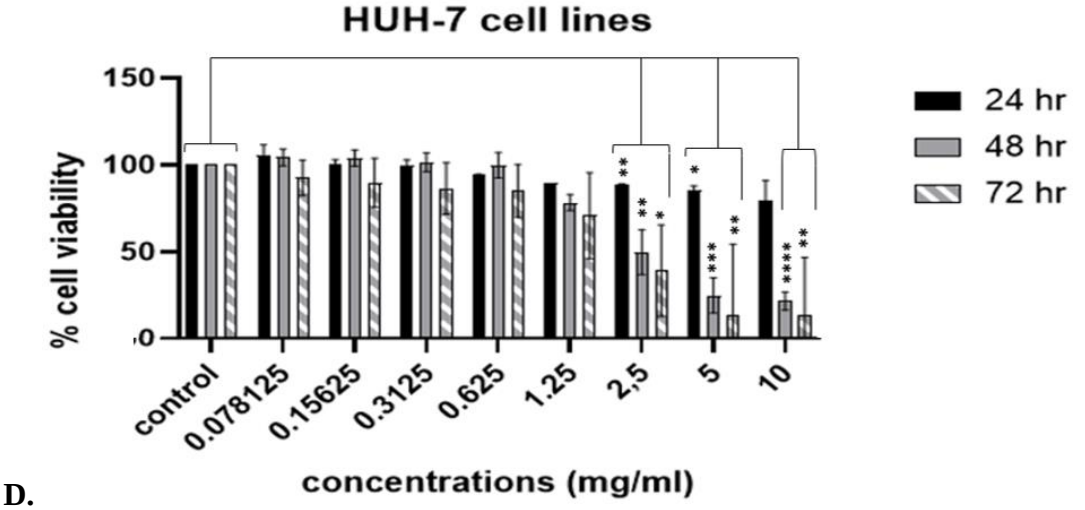


D.

Figure 4.1 Inhibitory effects of *Arum dioscoridis* L. plant seed extracts on cancer cell viabilities at different concentrations and times. Results of exposure to same extracts at

different concentrations and different times for a) A549 cell lines b) MCF-7 cell lines c) BEAS-2B cell lines and D) HUH-7 cell lines. As a result of the t test, the * value shown in the graph indicates: $p < 0.05$, ** value: $p < 0.01$, *** value: $p < 0.001$, **** value: $p < 0.0001$. If there is no sign (ns), it means $p > 0.05$.

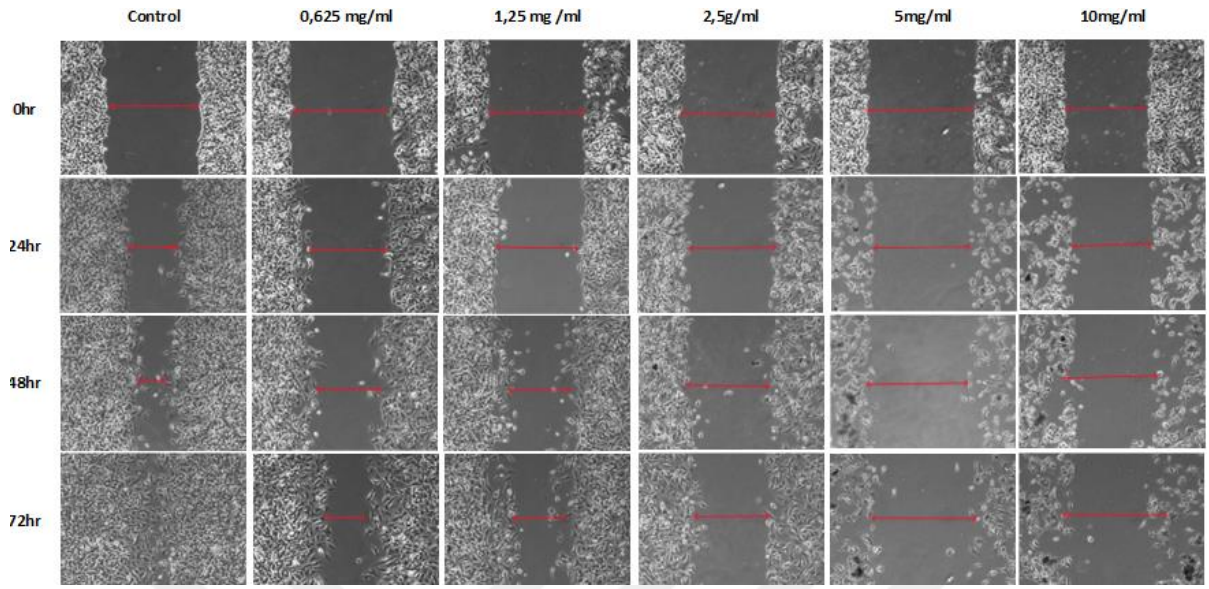
As a result of these analyses, it was seen that the viability of the cells was inhibited and in line with the results of other analyses, it was decided to use the A549 cell lines and the cells were dosed at the same concentration ranges.

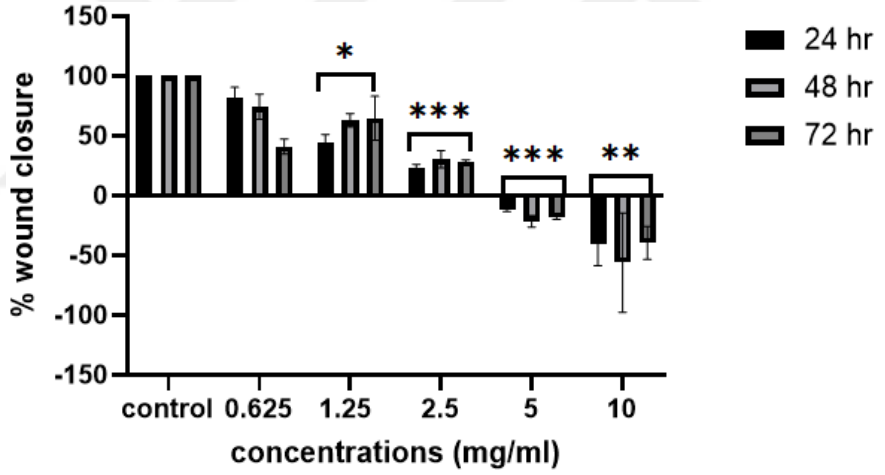
4.2. Wound Healing Assay

A wound healing test was performed to determine the wound closure and migration ability of *Arum dioscoridis* L. plant extract on the A549 cell line. In the test stages, a wound was made on the cells and *Arum dioscoridis* L. The extract was administered at concentrations of 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.625mg/ml, respectively. To see the effect on the cells over time, images were taken from the microscope from 3 different regions of the cells, upper, middle and dow, at intervals of 0, 24, 48, 72 hours (Figure 4.1A). Using the 0th hour as a reference, cell wound closure areas in all regions were calculated with the help of the ImageJ program. The wound closure area averages of the images taken from all regions were taken and the percentages of the wound closure areas were calculated and a graph was created with their changes over time (figure 4.1B).

As a result of the analysis, it was seen that as the concentration of the plant extract increased on the A549 cell line, the cells died and the cells lost their migration ability over time. It was found to kill cells at the highest concentrations (10mg/ml, 5mg/ml, 2.5mg/ml) and wound closure could not be observed.

A.





B.

Figure 4.2 : The effect of *Arum dioscoridis* L. extract on cell migration ability and wound healing capacity of A549 cell line. A) 10x images of the cells were taken with a microscope from middle part intervals of 0hr, 24hr, 48hr, 72hr. These images observed the effects of *Arum dioscoridis* L. extracts at different concentrations over time on wound closure. Concentrations was written on the photographs, the days was written next to them. B) Wound closure assays on A549 cells were calculated using the ImageJ program. The analysis showed the wound closure percentage at different concentrations over time.

4.3. The Effect of Hydromethanolic Seed Extract on Tumor Formation Potential of A549 Cell Line

A soft agar colony formation test was performed to determine the effect of *Arum dioscoridis* L. extract on colony formation abilities in the A549 cell line. In this experiment, cells suspended in 3D culture medium containing agar and agarose were treated with *Arum dioscoridis* L. extract at concentrations of 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.625mg/ml, respectively. The extract was applied to the cells every 3 days for three weeks. To determine the number of colonies, colony staining was performed by adding crystal violet dye to the colonies.

Colonies were counted with the ImageJ program. Colonies with 50 or more cells were counted. Using the control group as a reference, the findings showed that the colony-forming capabilities declined with increasing concentrations of *Arum dioscoridis* L. extract after three weeks of treatment in the A549 cell line. In this case, we can conclude that *Arum dioscoridis* L. extract suppresses the proliferation of cells on the A549 cell line.

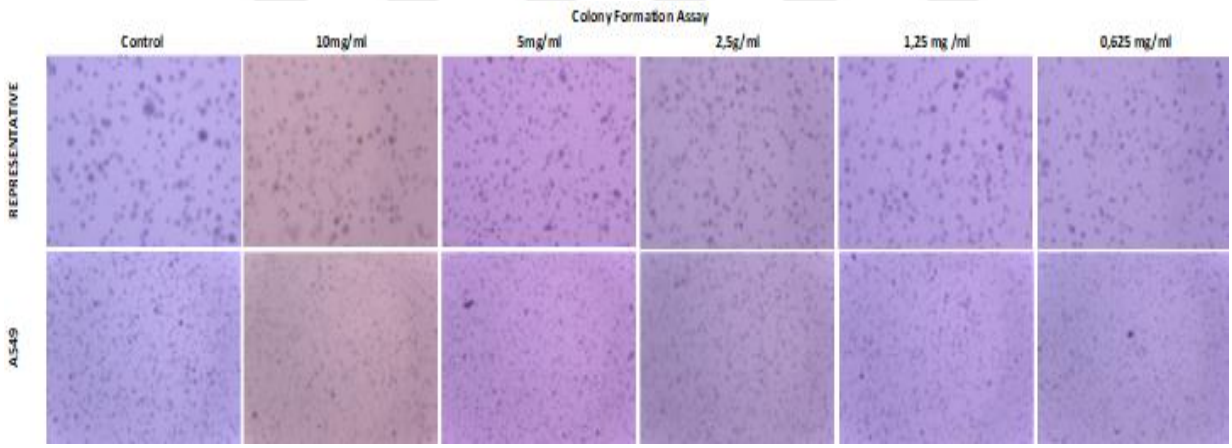


Figure 4.3 The effects of *Arum dioscoridis* L. plant seed extracts on tumor formation capacity of A549 cell line, the colonies were photographed under a dissecting microscope after crystal violet staining.

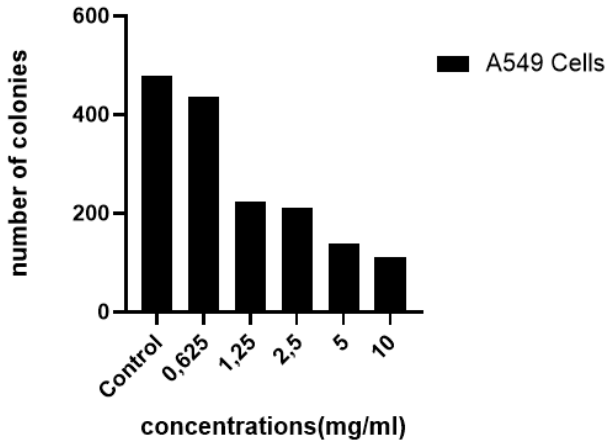


Figure 4.4. Graph of the number of colonies formed by A549 cells according to extract concentration increase.

4.4. Effect of *Arum Dioscoridis* L. Hydromethanolic Seed Extract on A549 Cells Apoptosis

With apoptosis analysis, in cells observed to be stained using Annexin V-FITC (green fluorescence) and non-vital dye propidium iodide (red fluorescence), living cells (FITCPI-), early apoptotic cells (FITC+PI-) and late apoptotic or necrotic cells (FITC+PI+) could be distinguished from each other according to the staining pattern. In DAPI and Annexin V-PI staining of A549 cells exposed to 10mg/ml, 5mg/ml, 2.5mg/ml, 1.25mg/ml, 0.625mg/ml hydromethanolic plant extract, cells incubated with dh20 instead of drug served as control in early stationary phase. As a result of the analysis, 10x, and 20x images were taken with a fluorescence microscope as in Figure 4.5.

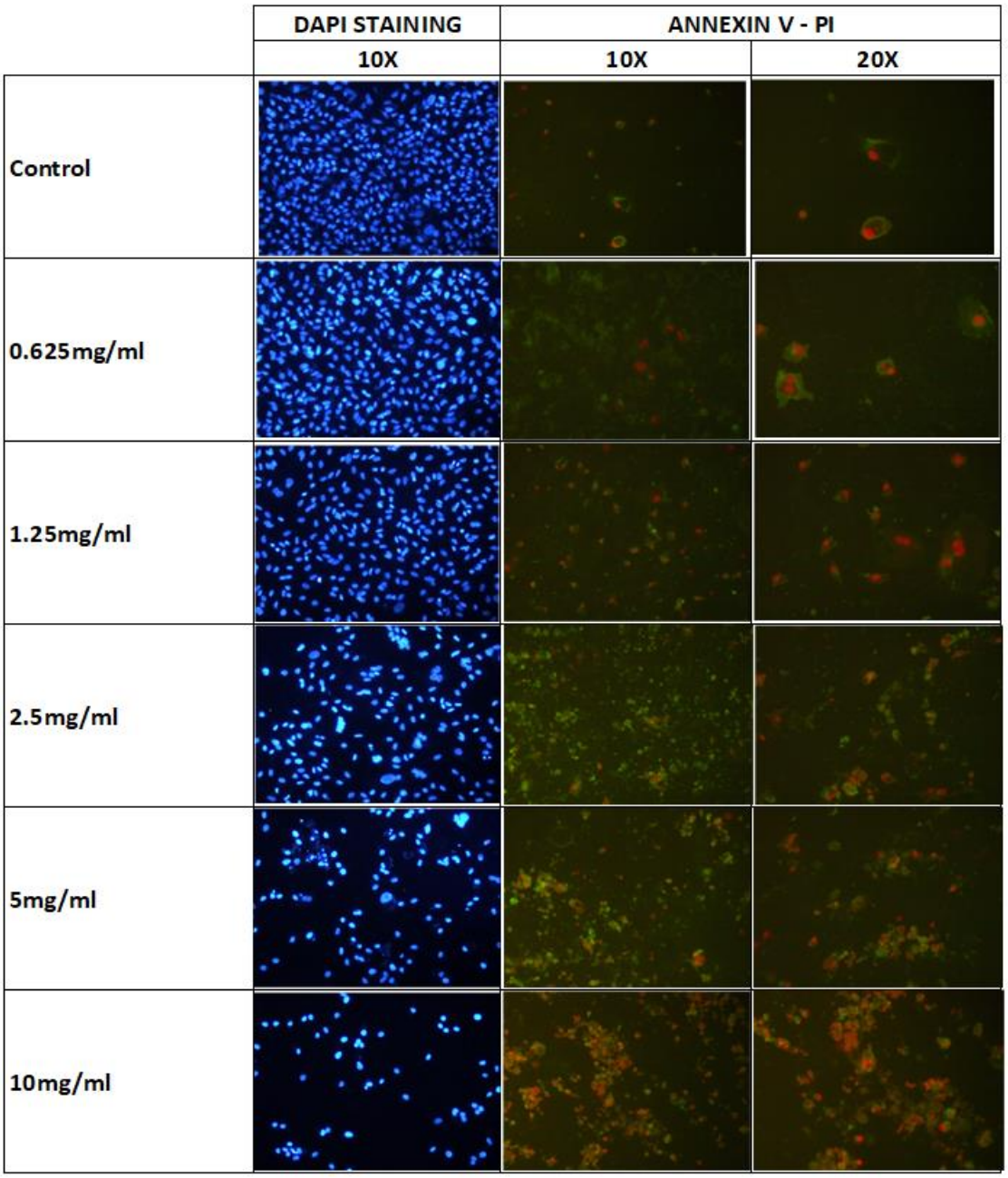
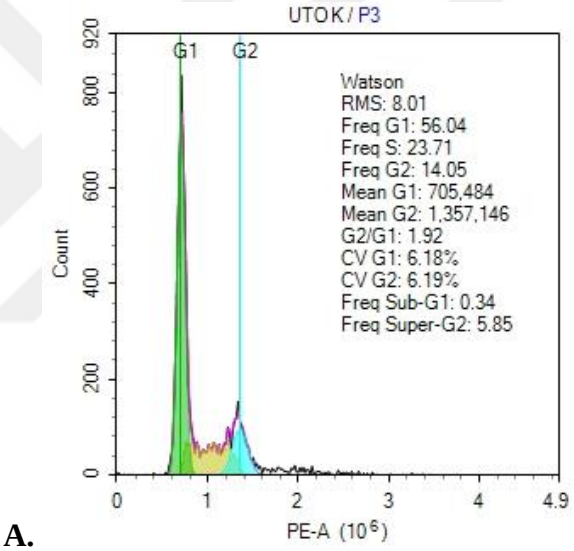


Figure 4.5. Flourescent microscopic analysis of apoptosis in extract treated A549 cells.

DAPI-stained A549 cells and exposed to different concentrations trigger apoptosis in a concentration-dependent manner. The red-colored cells observed on the side where AnnexinV and PI are stained represent dead A549 cells that died by necrosis. In the photographs, the cells that are stained green around the cell membrane are considered apoptotic or early apoptotic cells, while the yellow-stained cells (a combination of green and red) are considered necrotic or advanced apoptotic cells. These unstained cells are live cells.

4.5. The Effect of Hydromethanolic ic Plant Extract on A549 Cell Lines Cell Cycle Progression

Cell cycle analysis was performed after incubation of A549 cells with 2.5 mg/ml hydromethanolic ic plant extract for 48 hours. PI staining was performed and flow cytometry was used to DNA histograms of the cell cycle stages of A549 cells. As shown in Figure 4.6., percentages of sub-G1, G1, S, G2 and G2/G1 phases of the cells were analyzed by flow cytometry. DNA histograms were examined with cultures exposed to three different conditions: culture medium or media only, dH_2O + medium as control group, and 2.5 mg/ml hydromethanolic plant extract containing medium for treatment.



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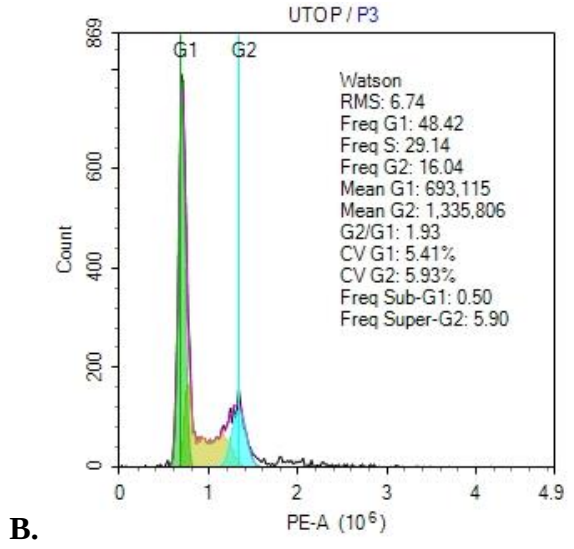

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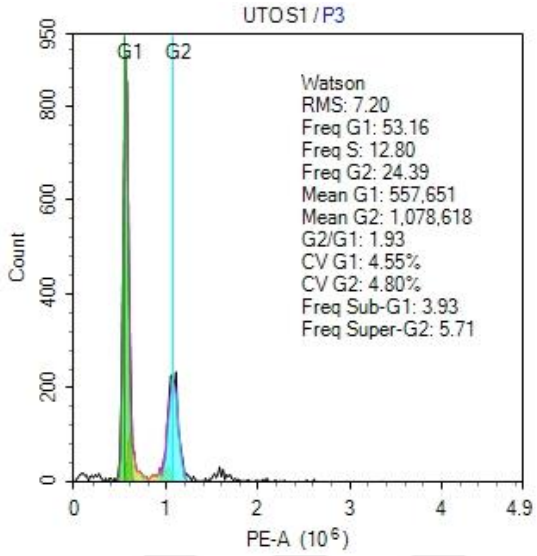

Figure 4.6. The effect of hydromethanolic plant seed extract on cell cycle progression in A549 cell line. A)media only treated cells B) d$\mathbf{H_2O}$ treated cells C) cells treated with 2.5 mg/ml hidromethanolic plant seed extract.

Table 4.1. The percent distribution of cell cycle stages after media only, dH_2O and 2.5 mg/ml hydrometanolic seed extract treatments

	% sub-G1	% G1	% S	% G2	% G2/G1
A549 full media	0,34	56.04	23.71	14.05	1.92
A549 control	0,5	48.42	29.14	16.04	1.93
A549 2.5mg/ml HMPE	3,93	53.16	12.80	24.39	1.93

.

As shown in Table 4.1, similar results were obtained in the cell fraction in full medium (%0.34) and control (%0.5), while an increase was observed in the 2.5mg/ml HPME (%3.93) analysis depending on the treatment medium. When the tested 2.5 mg/ml HPME effect was compared with the control groups, no decrease was observed in the data related to the G1 and G2 phases in A549 cells. In the G1 phase, Full medium is 56.04 %, control is 48.42 %, 2.5 mg/ml HPME is 53.16 %, and in G2 phase, Full medium is 14.05%, control is 16.04%, 2.5 mg/ml HPME is 24.39 %. In addition, 2.5mg/ml HPME treatment reduces the S phase cell population in A549 cells from 29.14% (control) to 12.8%.

5. DISCUSSIONS

Millions of people are diagnosed with cancer every year around the world, and most diagnosed patients die. According to the American Cancer Society, 2-3% of annual deaths recorded worldwide are due to cancer (Prakash et al., 2013). According to GLOBOCAN's statistics, the number of patients diagnosed with lung cancer in the world is more than 2.2 million, and there are approximately 1.8 million death records. Lung cancer is the most common cause of cancer deaths in the world (Jachowski et al., 2023). A patient diagnosed with cancer has various treatment options depending on the type of cancer and its progression. Treatment methods are then, determined depending on the patient's condition. Some patients may receive a single type of cancer therapy but most oftenly patients are administered with a combination of therapies (Ambroggi et al., 2015). The patient is usually given a mixture of chemotherapy, radiation, immunotherapy, surgery and hormone treatments. Many patients diagnosed with cancer seek alternative or complementary treatment methods due to the serious side effects of chemotherapy and radiation therapy (Shah et al., 2013). As a result, scientists are still investigating natural and efficient resources for cancer treatment (Teklehaymanot and Giday, 2007).

The anti-cancer properties of various plants are being brought to light, and many natural products and their analogs are being discovered to be potent anti-cancer agents (Prakash et al., 2013). These natural products have been used in cancer treatments for several years, and today many cancer drugs consist of natural compounds and their combinations. Many anticancer drugs exist, the most common examples of plant-derived molecules being taxanes (Paclitaxel and Docetaxel), vincristine and vinblastine alkaloids, podophyllotoxin derivatives (etoposide and teniposide), and camptothecins such as topotecan (Sak and Everaus, 2017). Nature provides a wide variety of compounds to combat tumor growth. Chemical modification of natural molecules can make them even more effective and at the same time reduce their negative effects (Cragg and Newman, 2005).

Considering the therapeutic properties of natural compounds, when the extract of *Arum dioscoridis* L., which grows locally in our country, was examined in different solutions, it was shown that the content of flavonoid substances such as isoorientin and vitexin was quite high (Afifi et al., 2016). The anticancer effect of the *Arum dioscoridis* L. plant, belonging to the *Arum* genus from the *Aracea* family, which has been found to have anti-cancer effects in

these natural components, was conducted against lung cancer cells in *in vitro* conditions on the A549 cell line. In this study, MTT cell viability assay was used to determine the effect on the viability of the cells; wound healing assay to determine the anti-migration ability of cells and soft agar colony formation assay was performed to observe the changes in proliferation and tumor formation ability of the cells.

Arum dioscoridis L. plants have been the subject of various studies because they are promising in terms of their flavonoid content. In these studies, the highest extraction efficiency was determined in extracts using highly polar solvents such as distilled water (27.38 %) and methanol (24.21 %). According to Uçan Türkmen et al. (2019), the extraction efficiency percentages for low polarity acetone and diethyl ether solvents were 9.5% and 8.60%, respectively. Considering this determination, MeOH and distilled water were used as solvents in the preparation of the plant extract obtained from *Arum dioscoridis* L. seeds to be used in our experiments.

It has been suggested that the natural components of many plants make them potent source for anti-cancer agents. The *Arum dioscoridis* L. plant contains flavonoids, such as isoorientin and vitexin, which indicate a potential for anti-cancer properties. A naturally occurring C-glucosyl flavone, isoorientin has a wide range of effects due to its many pharmacological properties (Ziqubu et al., 2020). Studies for isorientin have shown that it has antitumoral activity on lung, hepatocellular carcinoma, oral squamous cell carcinoma (OSSC), pancreas, and colorectal adenocarcinoma (Lin et al., 2016; S. C. Liu et al., 2021; Y. Xu and Qian, 2014). Vitexin is a flavonoid and lignan compound naturally found in various plant sources (*SCBT - Santa Cruz Biotechnology*, n.d.). Anti-cancer effects of vitexin *in vitro* were observed in breast adenocarcinoma (MCF7), bladder carcinoma (T24), cervical carcinoma (HeLa), colorectal carcinoma (HCT116andLoVo), malignant melanoma (A375, Sk-Mel-5, and Sk-Mel -28), glioblastoma (LN18) and lung cancer (A549) (Peng et al., 2021).

There are a limited nuber of studies using *Arum dioscoridis* L. plant extract. In the study conducted at Mersin University, the effects of the ethanolic extract of *Arum dioscoridis* L. alone and its combination with different concentrations of Tozasertib, a protein inhibitor of aurora kinase B (proteins responsible for centromere attachment of spindle fibers in the metaphase of cell division), on CFPAC-1 ductal pancreatic adenocarcinoma cells were investigated. In this study, whether the type of cell death was apoptotic or not, caspase 3, 8, 9 gene expressions involved in apoptosis, chromosomal transition complex (CPC) pathway

gene expressions involved in division, and VEGF gene expression levels effective in migration were examined. According to the study's findings, cells exposed to the ethanolic extract exhibited an increase in apoptosis (Ozturk, 2017). In a study conducted in Jordan using HT29, HCT116 colorectal cancer cell lines and the same extract, a significant cell death was observed at a concentration of 200 µg/mL, but no antiproliferative effect was observed at a concentration of 25 µg/mL (Afifi et al., 2016).

The changes in viable cell numbers of different cell lines in the presence of the plant extract were determined by MTT cell viability assay. Results showed that hydrometanolic plant extract can effectively reduce cell viabilities of all tested cell lines after 48 and 72 hours exposure. However, 24 hours exposure is also sufficient to avoid cell growth except MCF-7 cell line. Decreased cell viability in BEAS2B normal human bronchial epithelial cell line at high concentrations (>1.25 mg/ml) may suggest a more generalized cytotoxicity for tested extract. As a result, we can argue that *Arum dioscoridis* L. plant seed extract has an antiproliferative effect on all tested cell lines. Also, a time and concentration dependent consistent decrease in A549 cell line viability, deemed appropriate to test this cell line for further understanding of the nature of this anti-cancer effect.

The ability of cancer cells to move from one place to another in the body is called migration ability. Migration ability is the feature that plays the biggest role and the most dangerous aspect in the stages of metastasis and recurrence in tumor formation. To test the changes in the migration ability of cells in the presence of differing concentration of the plant extract, a scratch wound was made on the monolayer of growing A549 cell line. The amount of closure of the monolayer wound upon time was estimated as a reclection of the migration ability of the cells. Results showed that migration ability of A549 cells decreases in the presence of elevated concentrations of plant seed extract. This effect further intensified upon increasing time of exposure. Therefore, *Arum dioscoridis* L. plant seed extract may contain an antimigratory component as least against A549 cell line.

The ability of cells to proliferate and form colonies is an important ability for tumor formation and is a measure to test the level of aggresivity in cancer cells. After the cells migrate, they proliferate from a single cell source in a novel body part and form a colony. Therefore, the colony formation ability of *Arum dioscoridis* L. plant seed extract on A549 cells was examined in *in vitro* conditions. A549 cell line was treated with differing concentrations of the plant seed extract in an agar and agarose-based scaffold to immobilize the cells.

According to the results, as *Arum dioscoridis* L. extract concentration increases, colony formation ability of the cells decreases. It can be concluded that the extracts may suppress colony formation and prevent cells from forming new tumors.

Annexin V and PI signals were barely detectable in control cells, while in cells exposed to different concentrations of treatment medium, strong fluorescence intensities could be seen as the concentration increased. As a result, we can say that level of apoptosis in A549 cells are elevated as ~~its~~ extract concentration increased. In addition, in cells stained with DAPI stain, the proliferation of cells slowed down in parallel with the increase in concentration, and apoptosis was observed in existing cells. In other words, it was determined that *Arum dioscoridis* L. hydromethanolic plant extract initiated programmed cell death by inducing apoptosis in cells depending on the increase in concentration.

Flow cytometric analysis of cell cycle progression in tested conditions resulted in similar fashion in different cycle phases. However, an increase was observed in the cell fraction in the Sub-G1 phase in the treatment medium compared to the control. This may be attrıbuted as an indication of the activation of apoptotic processes in the cells. In other words, in the presence of the treatment medium, apoptosis might be triggered in the cells. Despite triggering apoptosis, the fact that the data did not change significantly in the G1 and G2 phases in either sample, may indicate that the extract has no effect on cell cycle progression.

6. CONCLUSION

Treatment methods for cancer, which is among the most common diseases in our country, are still being investigated vigoriously. Lung cancer is the leading cause of cancer deaths in the world. The most commonly used methods in cancer treatment are; chemotherapy, radiation, immunotherapy, surgery and hormone therapy. Natural compounds are preferred as an alternative to synthetic drugs due to their decreased side effects in clinical treatments. Plants have many natural compounds with anti-cancer properties that may serve as an alternative to current cancer treatments. Compounds with anti-cancer properties have been identified among the natural compounds in the *Arum dioscoridis* L. plants grown in our country.

In this work, I researched on further evidence for the potential use of natural compounds from these plants in cancer treatment. As a result, I found that *Arum dioscoridis* L. plant seed extracts can decrease cell viabilities in different cancer cell lines such as A549, MCF-7 and HUH-7. However, decrease in BEAS2B normal human bronchial epithelial cell line viability suggests a general cytotoxicity for this extract rather than a targeted effect on cancer cells. On the other hand, my studies on A549 small cell lung cancer cell line showed that migration and colony formation abilities can be decreased in the presence of the extract. Although cell cycle progression was not effected, a limited apoptosis can be triggered. Over all, this study recommends an anti-cancer potential for natural compounds from *Arum dioscoridis* L. plant seed extracts.

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