

Investigation of the Effect of *Allium polyanthum* on the ABCB1 Gene in Colon Cancer Treatment

Fulya KÖKTEN ^{1*}, Fevzi Berk ŞAHİN ¹, Hüseyin KEŞAN ¹, Gonca KABAK ², Yavuz SİLİĞ ²

¹ Sivas Cumhuriyet University, Faculty of Medical, Sivas

¹ Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Biochemistry, Sivas

*Corresponding author: fulyakokten@outlook.com

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Abstract

Colorectal cancer is one of the most common cancer types worldwide, presenting various challenges in treatment processes. This study investigates the potential effects of *Allium polyanthum* in colon cancer treatment, particularly focusing on its impact on the ABCB1 gene. The ABCB1 gene plays a significant role in developing resistance to chemotherapy drugs in cancer cells. In this research, in vitro colon cancer cell lines were utilized to examine the effects of *Allium polyanthum* extract on ABCB1 gene expression. Another crucial component of the study involves the evaluation of docetaxel, a commonly used chemotherapy drug in colon cancer treatment. Docetaxel belongs to the taxane class of drugs, effectively halting the division of cancer cells, but resistance development can limit treatment success. The results demonstrate that *Allium polyanthum* extract enhances docetaxel efficacy in colon cancer cells by suppressing ABCB1 gene expression.

Keywords: *Allium polyanthum*, colorectal cancer, drug resistance

1. Introduction

Cancer is characterized by the uncontrolled growth of abnormal cells resulting from genetic damage. It is the most common among somatic and genetic diseases. Environmental factors such as stress, radiation, and genetic factors play a role in cancer formation.

In our country, the most common cancers in men are lung cancer, while in women, breast cancer is the most prevalent. Colorectal cancer ranks third in both genders (Deniz, 2022). Among colorectal cancers, colon cancer is the most common in the gastrointestinal system (Gürbüz et al., 2015). The risk of colon cancer increases with age. Unlike other cancers, colon cancers arise from precursor lesions known as polyps (Remzi ve Öncel, 2006). Approximately 25% of asymptomatic patients have polyps, with 5% to 10% of these gaining invasive potential within 20 years.

Chemotherapy is one of the treatment methods used for cancer patients. Docetaxel, a herbal drug, is used to treat many cancer patients. Docetaxel is the active ingredient in several important drugs used in cancer treatment. The microtubule formed by docetaxel consists of an average of 13.4 tubulin subunits. Docetaxel is rapidly absorbed into cells and can remain inside cells for a long time. This property makes docetaxel significant in both in vivo and in vitro antitumor activity studies (Erdemoğlu ve Şener, 2000).

Garlic (*Allium sativum* L.) is a member of the Alliaceae family. It is known to have

originated in Central Asia and spread to China, the Middle East, the Mediterranean region, and subsequently to Europe (Singh ve Singh, 2008). Garlic contains various bioactive compounds such as organic sulfites, saponins, phenolic compounds, and polysaccharides, which contribute to its numerous health benefits (Bose et al., 2014; Alam et al., 2016; Diretto et al., 2017; Shang et al., 2019). Additionally, it is rich in vitamins, minerals, proteins, and fats. Garlic is one of the most important bulbous vegetables used as a spice and flavoring agent in food (Szychowski, 2018). It enhances the taste of food and aids in digestion. Garlic products are widely used as a source of medication in daily life and are considered a popular remedy for various ailments and physiological disorders. In recent years, numerous studies have revealed garlic's biological functions, including its antioxidant, cardiovascular protective, anticancer, anti-inflammatory, immunomodulatory, anti-diabetic, anti-obesity, and antibacterial properties (Seçkiner et al., 2014; Yun et al., 2014).

Allium polyanthum, also known as the many-flowered garlic, is a Mediterranean wild onion species native to Spain, France, Italy, Morocco, and Tunisia. Due to its edible and strongly aromatic bulbs and leaves, it is widely cultivated. *Allium polyanthum* typically produces an egg-shaped bulb surrounded by small bulbs at the base. The scape can grow up to 80 cm in height. The leaves are straight and broad, tapering to a point at the tip. The flowers are numerous and small, either white or pink (Anonim, 2024).



Figure 1. *Allium polyanthum* (Anonim, 2024)

ABCB1 (ATP Binding Cassette Subfamily B Member 1) is a gene that encodes a protein. As a member of the ATP-binding cassette (ABC) transporters' superfamily, it encodes an important protein. The multidrug resistance gene (*MDR1*), also known as the *ABCB1* gene, emerges after drugs are ingested (Leiri, 2012). The P-Glycoprotein encoded by the *MDR1/ABCB1* gene has substrate specificity, making it clinically significant (Marzolini, et al., 2004). This gene produces P-glycoprotein (P-gp), an ATP-dependent drug efflux pump with broad substrate specificity for xenobiotic

compounds. The P-gp encoded by *ABCB1* facilitates the transport of various drugs and phospholipids across cell membranes. This transport process is energized by ATP hydrolysis. P-gp can cause drug resistance in cancer cells by pumping various anticancer drugs out of the cells. This can reduce the effectiveness of treatment and make cancer treatment more challenging. *ABCB1* is an important transporter in the blood-brain barrier, protecting brain tissue from toxins and pharmacological agents. The role of *ABCB1* in drug resistance is of great interest in cancer treatment research.

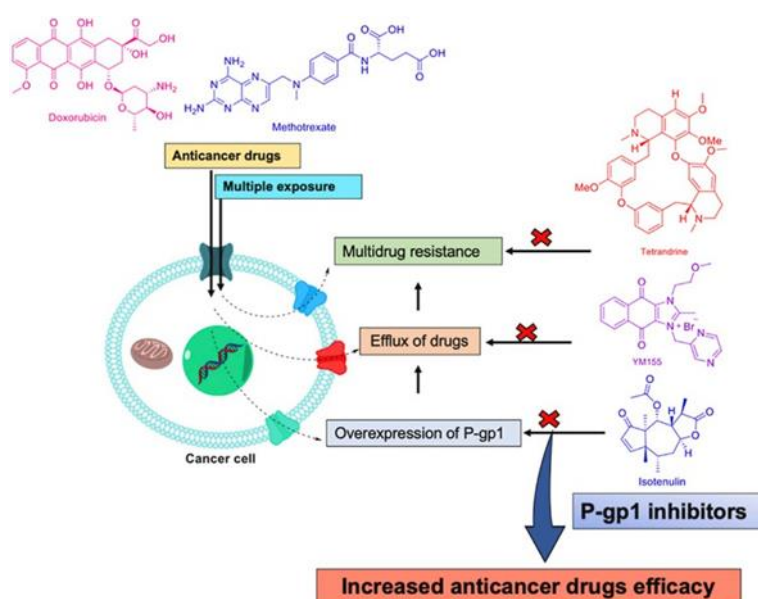


Figure 2. ABCB1 gene effect (Engle ve Kumar, 2022)

The expression of P-Glycoprotein in the gastrointestinal system and capillary endothelial cells of the blood-brain barrier leads to decreased oral absorption of many drugs, excretion from the body through urine and bile, and prevention of drug entry into the central nervous system (Londhe et al., 2011). Single nucleotide polymorphisms have been identified in MDR1 (Londhe et al., 2011). These polymorphisms are considered risk factors for many diseases, although their exact effect on P-Glycoprotein is not fully understood (Marzolini et al., 2004).

2. Materials and Methods

2.1. Plant material

Allium polyanthum Schult. plants used in this study were collected from Tepeköy and Ütük villages, located in the Zara district of Sivas province, Turkey, in May and September of 2023. We would like to express our gratitude to Assoc. Prof. Dr. Emre EVLİCE from the Department of Agricultural Sciences at Sivas University of Science and Technology for his support in the collection of the plant and the preparation of the extract.

2.2. Preparation of the extracts

The essential oils from the plants were extracted separately from the flowerless stem and flower+stem parts. The volatile oils were obtained by hydrodistillation of the specified parts of the plants using a Clevenger apparatus for 3 hours. The condenser part of the Clevenger apparatus was connected to a microcooler device to maintain the cooling water at 4°C. The yield of the obtained oil was determined as v/w (mL oil/g plant). The isolated volatile oil was transferred to amber-colored bottles and stored at

-20°C until analysis after being purified from water on Na₂SO₄ (Alkan et al., 2021).

2.3. Cytotoxicity activities

The IC₅₀ value found in the study conducted by Wang, Huang, et al. was utilized for cytotoxic activity assessment (Wang et al., 2016).

2.4. Cell culture

HT-29 and CCD18-Co cell lines were cultured in MEM and RPMI medium supplemented with 10% fetal bovine serum (FBS), penicillin (100 U/mL), and streptomycin (10 mg/L). The cells were maintained at 37°C in a humidified atmosphere containing 5% CO₂ and 95% air. Subsequently, cells were trypsinized from 70-80% confluent cell culture flasks and seeded into 6-well plates.

2.5. Statistical analysis

All experiments were conducted in triplicate, and the results were expressed as means ± standard error of the mean (SEM). Statistical analysis was performed using one-way analysis of variance (ANOVA), and differences were considered significant at $p < 0.05$. The IC₅₀ values were determined using statistical software, GraphPad Prism8 (GraphPad Software, San Diego, CA, USA).

3. Results and Discussion

Garlic's active ingredient, allicin, is a sulfur compound produced by consuming powdered garlic products obtained by crushing or chewing garlic (Lawson et al., 1992). Both in vitro and in vivo studies have shown that garlic potentially regulates the activity of P450 isozymes (Ameen et al., 2003). If these effects occur in patients treated for malignancy, garlic has the potential to disrupt the metabolism of many clinically important agents, including docetaxel, a CYP3A4 substrate drug (Marre et al., 1996). Based on this information, we aimed to investigate the effect of garlic on colon cancer cell lines in our study. The effects of *Allium polyanthum* extract and docetaxel on ABCB1 gene expression were examined. No significant change in gene expression was observed in cells treated with *Allium polyanthum* alone. However, a significant increase in expression was observed when combined with docetaxel. Firstly, the effects of active components of this plant on cells may be regulated by other factors in the cell environment.

Especially in cell culture conditions, the biological activity of the plant extract may be limited. The significant increase in expression of *Allium polyanthum* in combination with docetaxel indicates that the combined effects of these two agents are present. The use of *Allium polyanthum* in

combination with docetaxel, by enhancing the effectiveness of docetaxel, may play an important role in overcoming drug resistance of cancer cells. The use of *Allium polyanthum* with docetaxel may potentially increase chemotherapy effectiveness by increasing *ABCB1* gene expression.

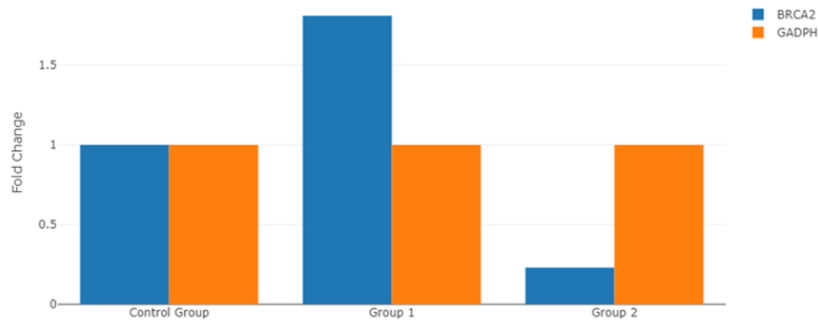


Figure 3. *ABCB1* Gene Levels in HT-29 Cell Line (The levels of *ABCB1* gene expression in the HT-29 cell line were assessed in two groups: Group 1, treated with *Allium sativum* L and Docetaxel; Group 2, treated with *Allium sativum*)

Table 1. Fold Changes in *ABCB1* Gene Compared to the Control Group

Symbol	Changes in fold induction	
	Group 1	Group 2
<i>ABCB1</i>	1.81	0.23
<i>GAPDH</i>	N/A	N/A

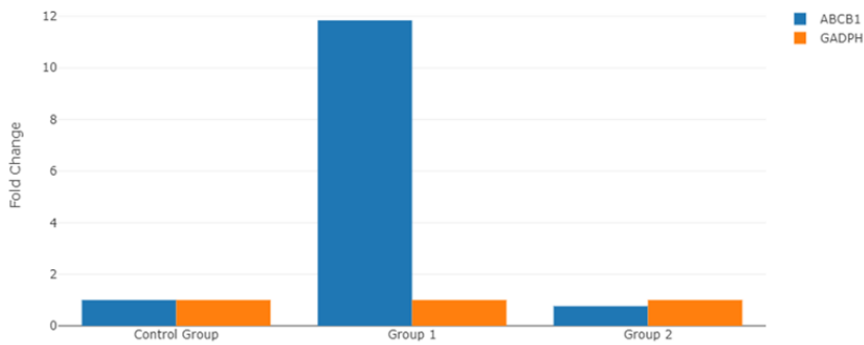


Figure 4. *ABCB1* Gene Levels in CCD-18Co Cell Line (The levels of *ABCB1* gene expression in the CCD-18Co cell line were assessed in two groups: Group 1, treated with *Allium sativum* L and Docetaxel; Group 2, treated with *Allium sativum*.)

Table 2. Fold Changes in *ABCB1* Gene Compared to the Control Group

Symbol	Changes in fold induction	
	Group 1	Group 2
<i>ABCB1</i>	0.32	0.41
<i>GAPDH</i>	N/A	N/A

4. Conclusion

In conclusion, this study demonstrates that *Allium polyanthum*, when used in combination with docetaxel, may enhance its therapeutic potential in the treatment of colon cancer. Future studies should further investigate the clinical efficacy and safety of this combination therapy in more detail. Such combinations of herbal and chemotherapeutic agents may contribute to the development of new treatments in cancer therapy.

Declaration of Author Contributions

The authors declare that they have contributed equally to the article. All authors declare that they have seen/read and approved the final version of the article ready for publication.

Declaration of Conflicts of Interest

All authors declare that there is no conflict of interest related to this article.

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