MAS Journal of Applied Sciences
Uygulamalı Bilimler Dergisi
ISSN: 2757-5675
masjaps.com

DOI: http://dx.doi.org/10.5281/zenodo.12167759
Araştırma Makalesi / Research Article

The Effect of Combined Treatment with *Mentha longifolia* L. and Docetaxel on Drug Resistance in Colon Cancer

Dilay AHMEDLİ [1], Zehra GÜMÜŞ [1*], Rana YETKİN [1], Gonca KABAK [2], Yavuz SİLİĞ [2]
[1] Sivas Cumhuriyet University, Faculty of Medical, Sivas
[2] Sivas Cumhuriyet University, Faculty of Medicine, Department of Medical Biochemistry, Sivas
*Corresponding author: zehragms2501@gmail.com

Received: 25.03.2024 **Accepted**: 30.04.2024

Abstract
This study investigates the effects of *Mentha longifolia* L. and the chemotherapeutic drug docetaxel on drug resistance in colon cancer. One of the major challenges in colon cancer treatment is the resistance of cancer cells to chemotherapy drugs. The primary mechanism of this resistance involves the overexpression of multi-drug resistance (MDR/ABCB1) genes. MDR genes reduce the intracellular accumulation of chemotherapeutic agents in cancer cells. The study examined the effects of *Mentha longifolia* L. extract and docetaxel on ABCB1 gene expression in the HT-29 colon cancer cell line. HT-29 cells were cultured in RPMI medium and CCD18-Co cells in MEM medium, both containing 10 % fetal bovine serum (FBS), penicillin (100 U mL^{-1}), and streptomycin (10 mg L^{-1}), at 37 °C, 5 % CO2, and 95 % humidity. Results showed that *Mentha longifolia* L. extract alone did not significantly alter ABCB1 gene expression, but in combination with docetaxel, it significantly increased gene expression. This finding suggests that *Mentha longifolia* L. could play a potentially important role in overcoming drug resistance in cancer cells by enhancing the efficacy of docetaxel.

Keywords: *Mentha longifolia* L., colon cancer, drug resistance

1. Introduction

Since the beginning of human history, diseases have been a part of life. People have sought treatments for these diseases in nature and through the experiences of previous generations. This quest has led to the development of traditional medicine, which has evolved over the years according to the beliefs, philosophical thoughts, and cultural structures of societies (Talhaoğlu, 2021). Traditional medicine utilizes plants, animals, and various minerals (WHO, 2000). Some methods used include prolotherapy, mesotherapy, and phytotherapy (Ünal and Dağdeviren, 2019).

Phytotherapy (phytos: plant, therapy: therapy) involves the treatment of diseases using plant- based remedies. Some of the plants used in phytotherapy include Aloe vera, tea tree oil, ginger, rosemary, and mint (Durusoy and Gözel Ulusal, 2007). The essential oil and leaves of mint are frequently used in traditional treatments and medicines due to their antioxidant, antitumor, antiallergenic, and antibacterial properties. Studies on animal models have shown that mint affects the gastrointestinal and nervous systems, has hepatic and renal effects, and possesses chemopreventive potential. In human studies, mint has been observed to affect drug and nutrient bioavailability, have gastrointestinal effects, alleviate airflow sensations in the respiratory tract, and possess analgesic effects (Diane et al., 2006). Phytotherapy methods can be used to address the severe side effects experienced by cancer patients undergoing treatment. Herbal treatments have also been observed to be effective in cancer immunotherapy, a modern approach to cancer treatment (Kurhan and Ekici, 2021). Cancer is a disease driven by genetic and somatic factors that cause uncontrolled cell proliferation. It can affect a tissue or organ and may metastasize, spreading to surrounding tissues and distant organs (Seyfried and Huysentruyt, 2013). Early diagnosis significantly reduces the risk of death, making cancer screenings crucial (Tekpınar et al., 2018). Globally, statistics indicate that cardiovascular diseases are the leading cause of death, with cancers being the second most common cause (Bayık, 1989). In Turkey, according to World Health Organization (WHO) screening tests, there are three primary types of cancer: breast cancer, cervical cancer, and colorectal cancers (Anonim, 2024). For breast cancer, mammography is recommended; for cervical cancer, pap-smear and HPV-DNA tests are suggested (Şen and Öztürk, 2020). For colorectal cancers, fecal occult blood tests, sigmoidoscopy, or colonoscopy can be used (White et al., 2015). Colonoscopy or sigmoidoscopy is particularly important for the detection and diagnosis of polyps, which are precursors to colon cancer (Hüseyin Remzi and Öncel, 2006).

Chemotherapy can be utilized to treat cancer based on its stage, to prevent invasion, and to hinder metastasis. One of the drugs commonly used in chemotherapy is docetaxel, a plant alkaloid-derived anticancer agent known for its anticancer effects through tubulin stabilization. The Mentha genus of the *Lamiaceae* family contains volatile oils widely used in the cosmetic and pharmaceutical industries. Following citrus species, Mentha species are the second most important source of volatile oils globally, with approximately 2000 tons of volatile oil obtained from Mentha species (Mucciarelli et al., 2001) Distributed primarily in temperate regions of Eurasia, Australia, and South Africa, where moist conditions prevail, this genus comprises over 25 species, mostly perennial herbs (Lange and Croteau, 1999) Among all species of mint, Mentha canadensis, Mentha piperita, and Mentha spicata are economically significant. M. canadensis (syn: Mentha arvensis L. f. Piperascens Malinv. Ex Holmes) is cultivated in tropical and subtropical climates, while M. piperita is suitable for temperate climates (Liu and Lawrence, 2007).

Figure 1. *Mentha Longifolia* L. (Anonim, 2023)

Commonly known as peppermint, M. piperita exhibits broad adaptability to various climates and soil conditions, with temperate climates being conducive to high-quality production (Green, 1963). It is extensively used for flavoring in pharmaceutical products and the cosmetic industry. In recent years, there has been a notable increase in international demand for peppermint oil, with Bulgaria, Italy, China, and the United States accounting for approximately 90% of the world's total production. The MDR1 gene, located on chromosome 7q21.12, is a multi-drug resistance gene. Some tissues, such as the epithelium of the colon and kidneys, express *MDR*.

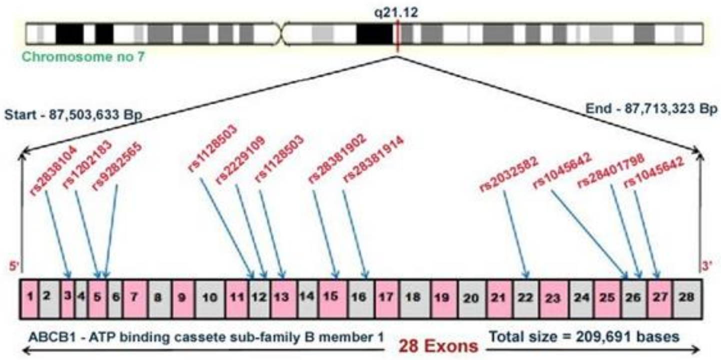

Figure 2. ABCB1 gene (Chakraborty et al., 2018)

While the exact physiological role of *MDR* remains incompletely understood, it serves as a substrate for numerous toxins, including natural anticancer drugs. *MDR* alters the uptake of these substrates in specific parts of the body and is involved in the elimination of drug effects. (Brinkhman and Eichelbaum, 2001; Jamroziak and Robak, 2004) Due to its role in causing multi-drug resistance in cancer, *MDR* can impede the success of chemotherapy. Therefore, efforts are underway to discover new drugs and enhance drug efficacy (Aksoy, 2016).

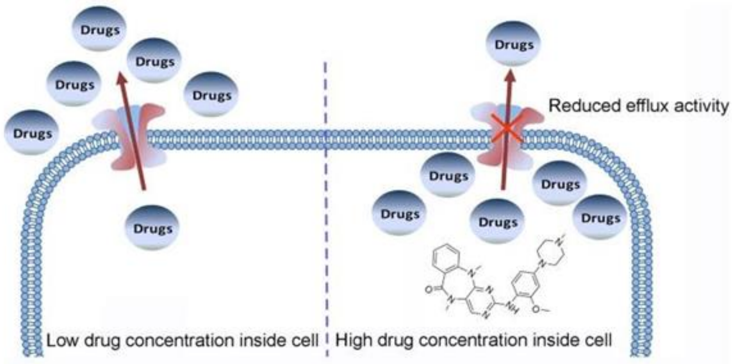

Figure 3. MDR gene effect (Wang et al., 2020)

2. Material and Methods

Mentha Longifolia L. used in this study was collected from Tepeköy and Ütük villages, located in the Zara district of Sivas province, Turkey, in May and September 2023.

2.1. Preparation of the extracts

Volatile oils from the whole plant without flowers and from the combination of flowers and stems were separately extracted. 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 micro-cooler 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 removing water content using Na2SO4.

2.2. Cytotoxicity activities

The IC50 value obtained from the study conducted by Beheshtian et al. was used for cytotoxic activity.

2.3. Cell culture

HT-29 and CCD18-Co cell lines were cultured in MEM and RPMI medium, respectively, supplemented with 10% fetal bovine serum (FBS), penicillin (100 U mL^{-1}), and streptomycin (10 mg L^{-1}). Cells were maintained at 37°C, 5% CO2, and 95% air in a humidified incubator. Cells were seeded in 6-well plates at approximately 70-80% confluence after trypsinization of cell culture flasks.

2.4. Statistical analysis

All experiments were conducted in triplicates, and results are presented as means ± standard error mean. Statistical analysis was performed using one-way analysis of variance (ANOVA), and differences were considered significant at $p < 0.05$. IC50 values were determined using GraphPad Prism8 software.

3. Results and Discussion

Generally, essential oils and aromatic substances used under medical supervision and at physiological doses are not toxic. However, misuse or uninformed treatment attempts can lead to toxic effects. Some aromatic compounds can be highly toxic, leading to their prohibition or restriction in the pharmaceutical industry, perfumery, and food flavors. For example, menthol, the main component of peppermint essential oil, can exhibit serious toxic effects when administered in high doses. Additionally, certain health conditions such as glucose-6-phosphate dehydrogenase deficiency (favism) can increase the toxicity of menthol. Therefore, the ability of essential oils (EOs) to inhibit cancer cell growth without harming healthy cells relies on their activation of specific molecular targets that trigger cell death. Moreover, some EO components have demonstrated the potential to shrink tumors with low toxicity and may be considered as a new class of anticancer drugs. Studies investigating the synergistic effects of EOs with other compounds, especially traditional drugs, are limited to in vitro conditions. Our study aims to increase the number of in vitro studies and enhance the response to treatment by demonstrating the combined effect of Mentha Longifolia L. and docetaxel drug in reducing resistance to drugs used in cancer treatment.

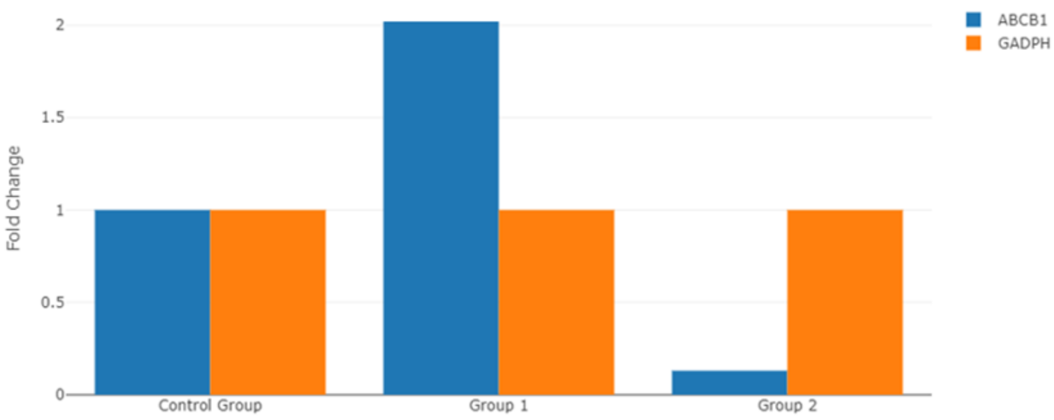

Figure 1. Levels of ABCB1 gene in HT-29 cell line (Group 1 = Treated with Mentha Longifolia L. and Docetaxel, Group 2 = Treated with Mentha Longifolia L. only)

Table 1. Fold changes of ABCB1 gene compared to the control group

Symbol	**Changes in fold induction**	
	Group 1	**Group 1**
ABCB1	**2.02**	**0.13**
GAPDH	**N/A**	**N/A**

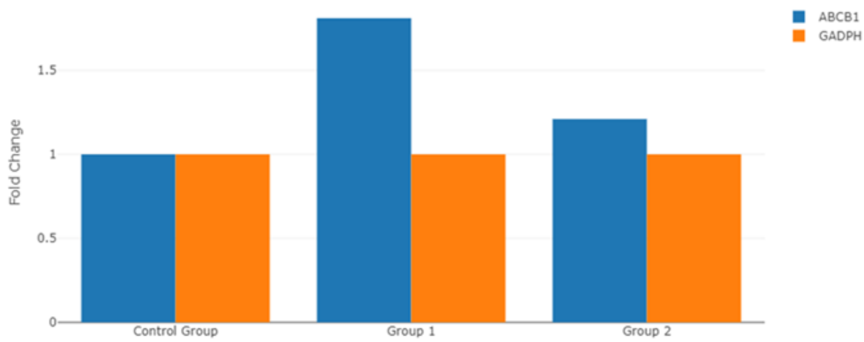

Figure 2. Levels of ABCB1 gene in CCD-18Co cell line (Group 1 = Treated with Mentha Longifolia L. and Docetaxel, Group 2 = Treated with Mentha Longifolia L. only)

Table 2. Fold changes of ABCB1 gene compared to the control group

Symbol	**Changes in fold induction**	
	Group 1	**Group 2**
ABCB1	**1.81**	**1.21**
GAPDH	**N/A**	**N/A**

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.

Acknowledgements

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.

References

Aksoy, Y., 2011. Ways to overcome drug resistance in cancer: Designing new drugs, *Journal of Medical Sciences*, 30(6).

Alkan, M., Güzel, M., Akşit, H., Bağdat, R.B., Alkan, F.R., Evlice, E., 2021. Chemical components and insecticidal effects of essential oils from three lavender cultivars against adult Sitophilus granarius (L., 1758) (Coleoptera: Curculionidae). *Turkish Journal of Entomology*, 45(4): 405-416.

Anonim, 2023. "*Mentha longifolia* (L.) L. | Plants of the World Online | Kew Science". Plants of the World Online. Retrieved 11-14.

Anonim, 2024. Turkish medical oncology association. (https://www.kanser.org/saglik/toplum/ kanser- tarama/ulkemizde-kanser-tarama-programlari), (Erişim Tarihi: 16.02.2024)

Bayık, A., 1989. Cancer epidemiology, *Journal of Ege University Faculty of Nursing*, 5(3): 58-71.

Beheshtian, N., Karimi, E., Asili, J., Beheshtin, N., Le, H.H., Shakeri, M., 2023. *Mentha longifolia* L. inhibits colorectal cancer cell proliferation and induces apoptosis via caspase regulation. *International Journal of Translational Medicine*, 3(4): 416-425.

Brinkmann, U., Eichelbaum, M., 2001. Polymorphisms in the ABC drugtransporter gene MDR1. *The Phanmacogenomics Journal*, 1: 59-64.

Chakraborty, R., Gupta, H., Rahman, R., Hasija, Y., 2018. In silico analysis of nsSNPs in ABCB1 gene affecting breast cancer associated protein P-glycoprotein (P-gp). *Computational Biology and Chemistry*, 77: 430-441.

Diane, L., McKay Jeffrey, B., Blumberg, A., 2006. Review of the bioactivity and potential health benefits of peppermint tea (*Mentha piperita* L.), *Wiley Inter Science*, 619-633.

Durusoy, Ç., Ulusal, B.G., 2007. Herbal therapy in dermatology-phytotherapy. *Turkish Journal of Dermatology*, 1: 47-50.

Green, R.J., 1963. MintFarming, Agriculture Information Bulletin No. 212. U.S. Department of Agriculture, Washington, DC, p. 26.

Jamroziak, K., Robak, T., 2004. Pharmacogenomics of MDR1/ABCB1 gene the influence on risk and clinical outcome of haematological malignancies, *Hematology*, 9(2): 91-105.

Kurhan, İ., Ekici, H., 2021. Complementary- phytotherapy and cancer approach under traditional medicine, *Bulletin of Veterinary Pharmacology and Toxicology Association*, 12(1): 15-20.

Lange, B.M., Croteau, R., 1999. Genetic engineering of essential oil production in mint. *Current Opinion in Plant Biology*, 2(2): 139-144.

Liu, W., Lawrence, B.M., 2007. Production of mintsandmintoil in China. In: Lawrence, B.M. (Ed.), Mint: GenusMentha. Taylor & Francis Group, Boca Raton, FL, pp. 165-184.

Mucciarelli, M., Camusso, W., Bertea, C. M., Bossi, S., Maffei, M., 2001. Effect of (+)-pulegone and other oil components of Mentha × piperita on cucumber respiration. *Phytochemistry*, 57(1): 91-98.

Remzi, F.H., Öncel, M., 2006. Screening and Surveillance for Colorectal Cancer, 17(1): 50-57.

Şen, S.K., Öztürk, Y.K., 2020. Relationship between health perception and cancer screening awareness, *Turkish Journal of Family Practice*, 24(4): 175-183.

Seyfried, T.N., Huysentruyt, L.C., 2013. On the origin of cancer metastasis, *Critical Reviews in Oncogenesis*, 18(12): 43-73.

Talhaoğlu, D., 2021. Traditional and complementary treatment practices. *Journal of Integravite and Anatolian Medicine*, 3(1): 16-29.

Tekpınar, H., Aşık, Z., Özen, M., 2018. Evaluation of the approaches of patients who applied to the Family Medicine outpatient clinic regarding cancer screenings. 22(1): 28-36.

Ünal, M., Dağdeviren, H.N., 2019. Traditional and Complementary Medicine Methods, *Eurasian Journal of Family Medicine*, 8(1): 1-9.

Valaes, T., 1994. Severe neonatal jaundice associated with glucose-6-phosphate dehydrogenase deficiency: pathogenesis and global epidemiology. *Acta Paediatrica*, 83: 58-76.

Wang, F., Li, D., Zheng, Z., Kin Wah To, K., Chen, Z., Zhong, M., Fu, L., 2020. Reversal of ABCB1-related multidrug resistance by ERK5-IN-1. *Journal of Experimental & Clinical Cancer Research*, 39: 1-13.

White, A., Thompson, T.D., White, M.C., Sabatino, S.A., Moor, J., Paul, V., Doria-Rose, Geiger, A.M., Richardson, L.C., 2015. Cancer Screening Test Use-United States, MMWR.

WHO, 2000. General Guidelines for Methodologies on Research and Evaluation of Traditional Medicine, WHO/EDM/TRM/2000.1

To Cite: Ahmedli, D., Gümüş, Z., Yetkin, R., Kabak, G., Siliğ, Y., 2024. The Effect of Combined Treatment with Mentha Longifolia L. and Docetaxel on Drug Resistance in Colon Cancer. *MAS Journal of Applied Sciences*, 9(2): 459–465.
DOI: http://dx.doi.org/10.5281/zenodo.12167759.