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Investigation of the anticancer and apoptotic effect of *Micromeria congesta* under *in vitro* conditions and detection of related genes by real-time PCR

Hikmet Dinç[1]*, Akın Yiğin[2], İsmail Koyuncu[3], Mustafa Aslan[4]

[1] Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Turkiye; [2] Department of Genetics, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Turkiye; [3] Department of Biochemistry, Faculty of Medicine, Harran University, Şanlıurfa, Turkiye; [4] Department of Educational Sciences, Faculty of Education, Harran University, Şanlıurfa, Turkiye.

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Abstract

At the present time cancer is one of the biggest health problems and because of the problems encountered in its treatment, alternative treatment methods of herbal origin are researched. In this study, the cytotoxic effects of the essential oil extracted from the *Micromeria congesta* plant on various cancer cells (A549, ECC-1, HCT-116, HELA, HGC-27, MDA-MB-231, SNU-423, U20S, DLD-1, PC-3) and normal cells (BEAS-2B, CRL-4010) have been examined. Anticancer mechanism of action has been particularly examined on gastric cancer (HGC-27; IC50: 15.84 µg mL^{-1}), on which essential oil showed a high cytotoxic effect. In the study, the cytotoxic effect and the apoptotic effect have been applied by MTT and flow cytometric annexin-V methods, respectively. The apoptotic gene expression (caspase 3, caspase 9, MMP2, MMP9, ACTB) real-time PCR content analysis has been performed with gas chromatography mass spectrometry (GC-MS). *M. congesta* essentials oil has the highest cytotoxic effect on gastric cancer (HGC-27) cells, decreases MMP2 and MMP9 expressions, and induces apoptosis with increasing the expression of caspase 3 and caspase 8 genes. In addition, it has been determined that piperitenone oxide (40.00 - 45.00%), pulegone (11.00%) and cyclohexanone (18.00 - 19.00%) are the major components of M. *congesta* essentials oil. In conclusion, it has been determined that the compounds found in high amounts in *M. congesta* plant induces apoptosis by affecting the expression of compound genes and thus can have the potential to be an alternative drug in the treatment of gastric cancer.

Introduction

Cancer is a fatal disease that occurs when cell proliferates and differentiates as a result of mutations and there is abnormal cell spread. Carcinomas account for 85.00% of cancers and originate from glands, skin and urogenital tissues (like prostate, cervix).[1] To prevent these diseases, the normal number of cells in the tissues and organs of multicellular organisms must be maintained. The equilibrium in the number of cells is achieved with new cell formation and a constant mortality rate.

Colorectal cancer orders forth among all cancers in terms of frequency of occurrence after breast, prostate and lung cancers and constitutes approximately 10.00 - 15.00% of cancers observed in men and women. Approximately 1 million new cases are diagnosed each year in the world and 500,000 deaths due to colorectal cancer are reported. According to the World Health Organization records, the number of new cases diagnosed every year is around 800,000.[2]

Today, chemical treatment is used most frequently in cancer treatment. The mechanism of action of anticancer drugs used in chemotherapy is not known precisely, but it is stated that they have a strong antiproliferative effects.[3-5] While these drugs have a toxic effect on the cancer cell, they also have a toxic effect on healthy cells and cause the destruction of the organs with the affected healthy cells. These undesirable side effects of anticancer drugs can result in death. In addition, it has been determined that resistance to anticancer drugs used in treatment has occurred in patients undergoing cancer chemotherapy. Resistance to anticancer drugs used in treatment has been

***Correspondence:**

Hikmet Dinç. PhD
Department of Pharmacology and Toxicology, Faculty of Veterinary Medicine, Harran University, Şanlıurfa, Turkiye
E-mail: hikmet.dinc@gibtu.edu.tr

determined in patients. In a study, chemotherapy improved 80.00% of patients with cancer, but 20.00% of patients were found to have cancer cells with developing resistance to chemotherapeutic drugs or creating fatal toxicity.[6]

For these reasons, the main methods and drugs used in cancer treatment are considered to be insufficient today. In recent years, alternative treatment methods are sought due to problems encountered in cancer treatment. In recent studies for this purpose, the anticancer effects of synthetic and herbal medicines against various types of cancer have been investigated. As a result of these studies, it has been suggested that secondary metabolites in plants should be used as an alternative method for the treatment of cancer. In many countries, especially in European countries, studies on the effect of different cancer cells are carried out intensively using plant extracts.[7-9]

Today there are many compounds of plant origin that have been proven to own apoptotic and antitumor effect and they are used for therapeutic purposes. The antimitotic taxol compound is obtained from the compound synthesized by a symbiotic living fungus in the bark of the *Taxus brevifolia* plant and currently used in the treatment of many cancer types - including cervix, lung, breast cancer and Kaposi's sarcoma in AIDS patients.[10] Campothecin which is synthesized from *Camptotheca acuminata* plant is a compound with anticancer activity, used in Chinese medicine,.[11] The herbal medicines, obtained via isolation from plants such as vincinistine, vinblastine, taxol, camptothecin and podophyllotoxin are successfully used in cancer chemotherapy.[12]

Many studies have been conducted on the anti-cancer activities of extracts of medicinal plants.[13-15] Compounds such as alkaloids, tannins, flavonoids and phenolics have therapeutic effects for human health.[16,17]

To determine the biological and pharmacological activities of plant extracts, the toxic or non-toxic effect on different cell lines must be examined. In these *in vitro* cyto-toxicity tests, the effects of compounds bearing drug property or having drug potential effects on the incubated cell culture are evaluated. With these tests, it is possible to analyze a large number of substances in a short time and the basic results can be translated on the animal experiments. There are numerous cytotoxicity tests with different mechanisms and sensitivities. Tetrazolium tests (MTT, MTS, XTT, WST) are mostly preferred by researchers.[3]

Traditional medicines obtained from such plants have an important place in the treatment of diseases as they have fewer side effects compared to synthetic drugs and the synergistic effect of the compound combination.[18,19]

Biological efficiency, quality standardization and prevention of contamination by external factors are very important for the plants used in these studies. In addition, to determine the content analysis of chemicals extracted, it is necessary to examine and register those in herbarium.

Lamiacea member plants are widely grown in the mountainous areas of the Mediterranean, Eastern and Southeastern Anatolia regions. It has been known that there are 14 *Micromeria* species and their 22 taxa in Turkiye's flora and 12 of these grow endemically. The *M. congesta* grows naturally in the mountainous areas of Şanlıurfa, Adıyaman and Gaziantep and is locally known as 'Kaya Yarpuzu', 'Gihayepaluk' and 'Pungetehta'. It has been reported that the decoction of aerial parts of the plant has been used by local people for treating respiratory diseases and cough. It has been stated that the essential oil extract obtained from the plant bear antimicrobial, antioxidant, anti-inflammatory, antiseptic, antirheumatic MSS stimulant properties and is used as analgesic for tooth aches.[20-24]

The antimicrobial and antioxidant properties of *M. congesta* have been studied previously, but cytotoxic and anticancer activities have not been studied. In this study, we aimed to determine the anticancer effects of the essential oil obtained from *Micromeria congesta* Boiss. et Hausskn. ex Boiss plants, which are traditionally used for medical purposes in the treatment of various diseases in our region, *in vitro.*

Materials and Methods

Plant material. The essential parts of *M. congesta* were collected at Germüş village (37° 12' 2" N, 38° 51' 12" E; altitude 550 m) in Şanlıurfa province in July-August 2018 during the flowering period. The identification of the collected plants was made by Dr. Mustafa Aslan and stored in Harran University herbarium with the number 4521. Samples were air dried - and stored in polyethylene bags until used. The essential oils of the dried parts were obtained by hydrodistillation using the Clevenger apparatus. The essential oil was extracted by hydro-distillation using a modified Clevenger apparatus coupled to a 2.00 L round-bottom flask. 40.00 - 150 g of dried aerial parts of the plants were cut into small pieces and then subjected to hydrodistillation for 8 hr using a Clevenger-type apparatus. The essential oils were collected and stored in sealed vials protected from the light at –18.00 ˚C before analysis. The essential oils of species were diluted by dichlorometane (1:3, v/v) before the gas chromatography run. The yield of the essential oil of *M. congesta* on a dry weight basis was 3.20% (v/w). The oil was colorless. The oil samples were dried over hydrous sodium sulfate and allowed to stand at 4.00 ˚C until analysis. [25-27]

The specifications of gas chromatography mass spectrometry (GC-MS) device. The GC-MS analysis for determining the chemical content of essential oil extract was conducted in Shimadzu brand (Shimadzu Corporation, Kyoto, Japan) device owning TRB-WAX (100% polyethylene glycol) column (0.25 mm × 30.00 m, 0.25 µm film layer thickness) and electron impact (EI) detector. In the analysis, the column temperature was

increased from 50.00 ˚C to 160 ˚C with an increase rate of 3.00 ˚C per min and waited 10 min at this temperature and then temperature was increased to 260 ˚C again with an increase rate of 3.00 ˚C per min using Helium at a flow rate of 1.00 mL per min as the carrier gas. The sample volume, the injection volume and the ionization voltage were set to 2.00 mL, 1.00 µL and 70.00 eV, respectively. Mass Range was kept at 41.00 - 400 Mw. Quantitative amount were determined with calculating peak areas. The extracted compounds were identified by the method of comparison with the data in the mass spectral library of the International Standards and Technology Industry.

Cells and culture conditions. In this study, human prostate (PC-3), adenocarcinomic human (A549), endometrial cancer (ECC-1), human colon cancer (HCT-116, DLD-1), cervival cancer (HELA), gastric cancer (HGC-27), breast cancer cells (MDA-MB 231), hepatocellular carcinoma (SNU 423), osteosarcoma (U20S) and normal epithelial (BEAS-2B, CRL-4010) cell lines were used which were obtained and stocked from ATCC (Manassas, USA). Cell lines were fed and proliferated in DMEM (Thermo Fisher Scientific, Waltham, USA) F12 and RPMI-1640 (Thermo Fisher Scientific) petri dish that was supported with 10.00% FBS (Merck, Darmstadt, Germany) and 1.00% glutamine (Sigma-Aldrich, St. Louis, USA). All cells were incubated at 37.00 ˚C in a 5.00% CO_2 atmosphere. Cells were removed with 0.25% trypsin, (Thermo Fisher Scientific) 0.03% EDTA (Merck) and passaged at a 1:2 or 1:3 ratios as suggested by ATCC, unused cells were stored in 95.00% petri dish and 5.00% Dimethyl sulfoxide (DMSO; Merck), at –80.00 ˚C deep freezer for short-term or long-term, in liquid nitrogen.

Determination of cytotoxic effect of *M. congesta* essential oil by MTT assay. Cytotoxic effects of *M. congesta* essential oil were examined using MTT method. The MTT test is done via basing on the principle of breaking down the metabolic activity in the living cells by mitochondrial dehydrogenase enzyme to form soluble formazan salts. Cells were opened from stock and planted into 25.00 cm^3 flasks, removed by trypsinization at 80.00 - 90.00% and then planted to 96-well sterile plates, for populating 1.00 × 10^4 cells per well. After 24 hr period, 5-FU (Pubchem, Bethesda, USA) and *M. congesta* oil were dissolved with DMSO and applied to each extract and cytotoxic dose was tried to be found. Plant extracts were added to each well with 200 µL volume and then incubated in a 37.00 ˚C oven in a 5.00% CO_2 atmosphere for 48 hr. After the incubation period, the nutrient in the wells were removed and 90.00 µL MTT solution was added on to 20.00 µL serum-free petri dish and allowed to incubate for an average of 4 hr. At the end of the incubation, the nutrient on plates was removed and after the formazan crystals were dissolved with 100 µL DMSO, the absorbance values were measured by spectrophotometer at 570 - 690 nm wave length. The difference between the mean value of the wells with cells and the mean values of wells without cells were measured and the percentage cytotoxicity was calculated by applying the formula. The dose which killed 50.00% of the culture (IC_{50}) was calculated from the curve of the graph plotted according to the readings.

Apoptosis analysis (flow cytometry). Annexin-V-FITC test was used to examine the essential apoptotic effects. As a result of cytotoxic examination, IC50: 15.84 apoptotic efficacy of upper doses. The *M. congesta* essential oil extract was added to the cells in 25.00 µg mL^{-1} and 50.00 µg mL^{-1} doses and then the cells were incubated for 48 hr for performing apoptotic analysis using FITC annexin-V Apoptosis Detection Kit I (Becton Dickinson Biosciences, Franklin Lakes, USA) according to the manufacturer's recommendation. Briefly, the cells were centrifuged at 1,200 rpm for 5 min and suspended in tube with 1X binding buffer. Then 5.00 µL of fluorochrome-conjugated annexin-V and 5.00 µL of Propidium Iodide color or 7-Amino-Actinomycin D (7AAD) were added and incubated in the dark for 15 min at room temperature. After the incubation period, the percentage of apoptotic cells at the end of incubation period was examined by flow cytometry (BD Biosciences) analytical method.

Molecular genetic analysis. The gene expression analysis was performed by real-time PCR, using three groups, each containing eight samples. *M. congesta* (*M. c* HGC-27) and 5 fluorouracil (5-FU PC-3) were applied to the HGC-27 cell lines. Only HGC-27 was used as control group cells. RNA isolation was performed with 1.00 × 10^6 PC-3 cells for each sample in the group, in compliance with the High Pure RNA isolation kit protocol (Roche Diagnostics, Basel, Switzerland). The RNA purity and integrity were checked by NanoDrop spectrophotometer (Nanodrop 2000; Thermo Fisher Scientific) gel electrophoresis (Mini-PROTEAN® Tetra Cell; Bio-Rad, California, USA) cDNA extract was performed according to the cDNA Transcriptor First Strand cDNA synthesis kit procedure (Roche Diagnostics). The cDNAs extract was obtained by keeping this mixture in Thermal Cycler at 25.00 ˚C for 10 min, at 50.00 ˚C for 60 min and at 85.00 ˚C for 5 min. Primers for target and reference genes were designed by IDTDNA primer design software (Table 1).

Table 1. Genes expressions primers sequences

Oligonucleotide ID	Nucleotide Sequence
Caspase 3	5'-GCTGCCTGTAACTTGAGAGTAG -3' 5'- GTATGGAGAAATGGGCTGTAGG - 3'
Caspase 9	5'- GAGGAAGAGGGACAGATGAATG -3' 5'- CATGTCAGTAGTGCAGAGGTT -3'
MMP-2	5'- TGCTGAAGGACACACTAAAGAA -3' 5'- CGCATGGTCTCGATGGTATT-3'
MMP-9	5'GTG CTG GGC TGC TGC TTTGCT G 3' 5' GTC GCC CTC AAA GGT TTGGAA T 3'
ACTB	5'-CTGGAACGGTGAAGGTGACA-3' 5'-CGGCCACATTGCAGAACTTT- 3'

In the real-time PCR study, the primer sequences were designed and used for the target genes caspase 3, caspase 9, MMP2, and MMP9 together with ACTB (Beta actin) reference genes. The study was conducted with the PCR mixing protocol having a total mix volume 18.00 µL, consisting of 12.40 µL ddH_2O, 1.60 µL Mg^{+2} (25.00 Mm), 2.00 µL Light Cycler DNA Master SYBR Green I 10X concentration, 1.00 µL Primary Forward (Target or ACTB), 1.00 µL Primary Revers (Target or ACTB), and then 2.00 µL of each Target cDNA or ACTB was added on top of this mixture for reaching a total ultimate concentration volume of 20.00 µL. The real-time PCR protocol was performed with one cycle at 95.00 ˚C for 30 sec, 45 cycles at 95.00 ˚C, at 60.00 ˚C for 30 sec, at 72.00 ˚C for 20 sec and one cooling cycle at 40.00 ˚C for 30 sec. The study was conducted with Qiagen Rotor-Gene® Q real-time PCR device (Qiagen, Hilden, Germany) by adding 5.00 µL cDNA to above mixes for each sample.

Statistical analyses. The results were expressed as mean ± standard deviation. The spectrophotometric measurement results obtained as a result of the MTT method applied for the cytotoxicity determination of HGC-27 cells were evaluated statistically with the Student's *t* test using the Graph Pad Prism (version 5.0; GraphPad software Inc., San Diego, USA) . Significance value was accepted as $p < 0.05$.

Results

Gas chromatography mass spectrometry. As a result of the analysis of the essential oil extract obtained from dried *M. congesta* plant, the major components of the chemical structure were found to be piperitenone oxide (40.00-45.00%), pulegone (11.00%) and cyclohexanone (18.00 - 19.00%), (Fig. 1).

Cytotoxic activity. After examining the cytotoxic effects of *M. congesta* essential oil extract on A549, ECC-1, HCT-116, HELA, HGC-27, MDA-MB-231, SNU-423, U20S, DLD-1, PC-3, BEAS-2B and CRL-4010 cells by MTT method, IC_{50} (cytotoxic 50:50% cell death) values were determined and all results were plotted and shown in Figure 2A.

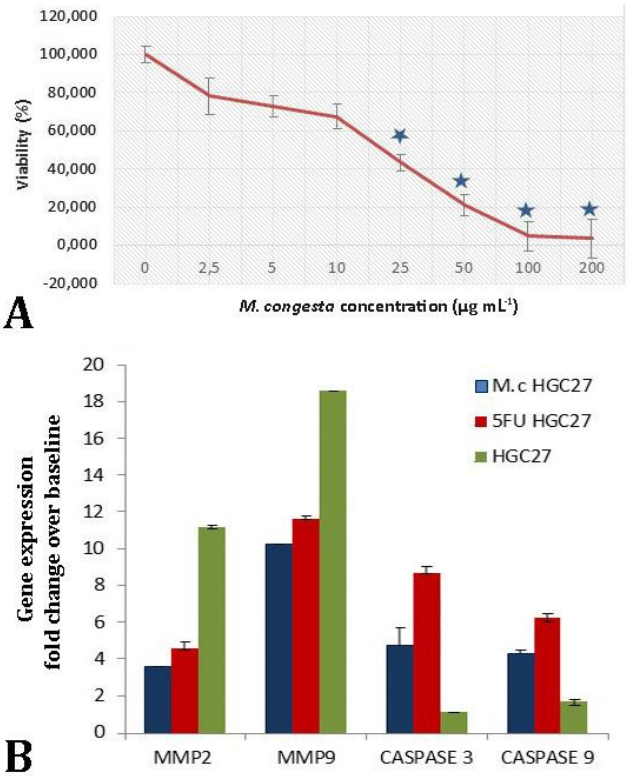

Fig. 2. A) Investigation of the cytotoxic activity of *M. congesta* essential oil on HGC-27 cells by MTT method (µg mL^{-1} as mean ± SD). * Statistical significance was evaluated unilaterally compared to the control group ($p < 0.05$); **B)** *M. congesta* gene expression fold change data in HGC27 cell line.

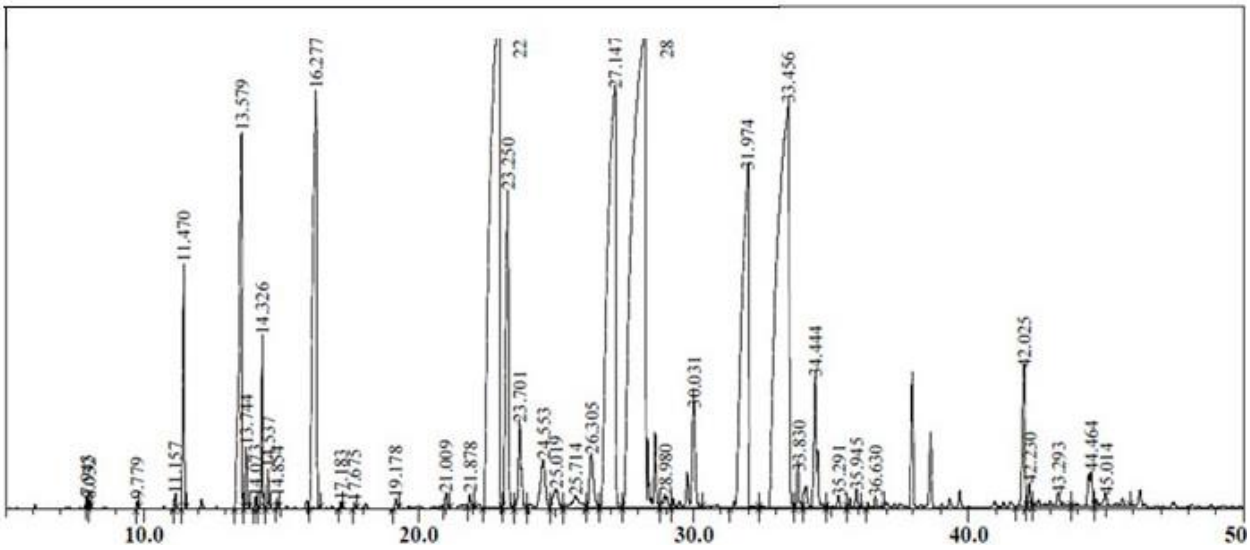

Fig. 1. Gas chromatography mass spectrometry analysis results of *Micromeria congesta* essential oil.

It was observed that the essential oil extract obtained from *M. congesta* plant significantly reduced the viability of cancer cells. It was found out that the extracted compounds showed different doses of cytotoxic effect on cancer cells. It was determined that among these concentration levels, *M. congesta* showed the strongest cytotoxic effect on HGC-27 cells at 50.00 µM dose level (IC_{50}: 15.84 µM), (Table 2). It was also determined that the cytotoxic effects of *M. congesta* compound on the other cancer cells was very low compared to the cytotoxic effects on healthy cells. Therefore, the molecular mechanism of the essential oil extract of *M. congesta* plant on cancer cell (HGC-27) gastric cancer cells, which was found to have the strongest cytotoxic effect among 12 cancer cells was examined.

Table 2. Cytotoxic effects of *M. congesta* essential oil extract on the cancer and normal cell line (IC_{50}: µg mL^{-1}).

Cell lines	*M. congesta*	5-FU
A549	65.19 ± 12.50	23.90 ± 5.20
BEAS-2B	15.91 ± 2.30	24.80 ± 4.30
CRL-4010	39.48 ± 11.30	133.70 ± 11.20
ECC-1	29.22 ± 5.60	32.80 ± 10.30
HCT-116	38.71 ± 5.63	69.90 ± 10.70
HELA	42.94 ± 8.20	14.00 ± 1.20
HGC-27	15.84 ± 1.60	2.20 ± 0.80
MDA-MB-231	27.46 ± 4.20	11.10 ± 3.20
SNU-423	31.68 ± 7.30	44.40 ± 3.20
U2OS	134.86 ± 18.30	118.10 ± 19.20
DLD-1	29.64 ± 10.20	55.40 ± 14.80
PC-3	24.14 ± 2.30	67.60 ± 10.50
CRL-4010	99.59 ± 9.85	45.30 ± 15.20
HELA	37.68 ± 6.20	24.20 ± 5.90

Apoptosis analysis. The anticancer activity levels of natural extracts obtained from medicinal plants depends on their ability to cause cancer cell death by triggering apoptosis. In our study we conducted cell cytotoxicity experiments using 25.00 µg mL^{-1} and 50.00 µg mL^{-1} concentrations of *M. congesta* essential oil extract compound and 5-FU as the positive control. In addition the death rate of HGC-27 cells that were 50.00 µg mL^{-1}, dosed and incubated for 48 hr, and was also analyzed with flow cytometric by annexin-V Apoptosis/Necrosis analysis method for uncovering whether the cell deaths were mainly apoptotic cell death or not.

For each sample, 10,000 cell counts were performed on flow cytometry. Annexin-V (-) and PI (-) cells are healthy, annexin-V (+) and PI (-) cells were early apoptotic, annexin-V (+) and PI (+) cells were late apoptotic and finally annexin-V (-) and PI (+) cells were considered to be necrotic (Fig. 3, Table 3). When apoptotic / necrotic cell death rates of two different doses of essential *M. congesta* applied group were compared to control group, apoptotic cell death was observed in both doses. At 25.00 µg mL^{-1} dose, it was found that early apoptosis was 17.20%, late apoptosis was 8.80%, necrosis was 6.10% and apoptotic effect was increasing with increasing dose. It was found that the 50.00 µg mL^{-1} dose level caused a high rate of apoptosis. Apoptotic effect was mostly in late apoptosis stage (37.00%) and the percentage of late apoptosis stage was increasing (8.80-37.00%) with dose increase (Table 3). It was determined that the number of living cells was decreased due to the increase in dose in the group applied in *M. congesta*, while an increase in the number of cells undergoing apoptosis was observed. Compared to the negative group and the number of live cells was decreased significantly ($p < 0.05$) in the group applied in *M. congesta.* There was a significant increase in the number of cells apoptosis ($p < 0.05$). It was observed that the application of 50.00 µg mL^{-1} *M. congesta* was very close to the positive (5-FU) group of cells of late apoptosis. As a result, it was determined that *M. congesta* essential oil had an effect close to 5-FU used in chemotherapy. When the related gene expressions of HGC-27 cells were examined, it was shown that decreased MMP2, MMP9 expressions and increased the expression of caspase 3 and caspase 8 genes in HGC-27 cells by increasing apoptosis (Fig. 2B). The MMP2 and MMP9 genes are the genes that encode an enzyme that breaks down the type IV collagen, which is the main structural component of the basal membranes. The caspase 3 and caspase 9 genes are important genes involved in apoptosis. These genes were upregulated 3 times for caspase 3 and 2 times for caspase 9 compared to the control group (HGC-27) with a statistically significant difference ($p < 0.05$). However, the group (5-FU HGC-27) was higher than the other two groups, indicating that *M. congesta* caused the cells to undergo apoptosis although not as effective as 5-FU.

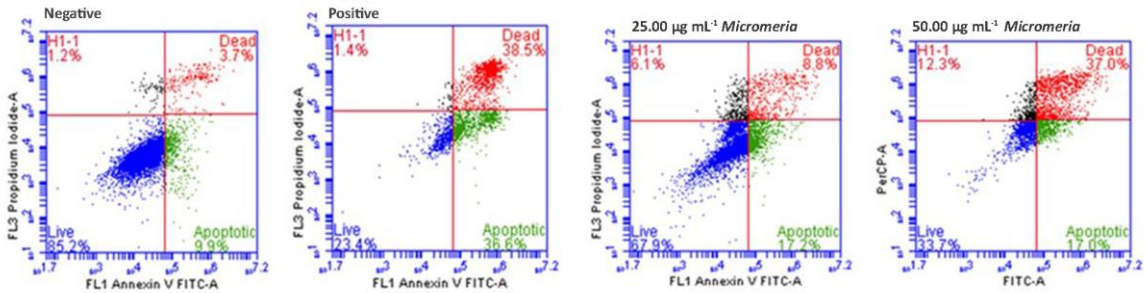

Fig. 3. Induction of apoptosis in HGC-27 cells by *M. congesta* and 5-FU The apoptosis ratio was analysed by flowcytometry. Data analyses of HGC-27 cells and contour diagram of annexin-V/PI flow cytometry.

Table 3. Apoptotic effects of *M. congesta* essential oil extracts on the HGC-27 cell line. Data are presented as mean standard error of the mean (n = 3).

Groups	Live (%)	Early apoptosis (%)	Late apoptosis (%)
Negative	85.20 ± 15.20	9.90 ± 3.50	3.70 ± 1.20
5-FU (25.00 µg mL^{-1})	23.40 ± 2.36^{a}	36.60 ± 7.90^{a}	38.50 ± 6.50^{a}
***M. congesta* (25.00 µg mL^{-1})**	67.90 ± 10.10^{ab}	17.20 ± 5.60^{ab}	8.80 ± 2.35^{ab}
***M. congesta* (50.00 µg mL^{-1})**	33.70 ± 4.50^{ab}	17.00 ± 4.20^{ab}	37.00 ± 7.60^{a}

^{ab} indicate statistical significance compared to the negative and 5-FU groups, respectively ($p < 0.05$ for both comparisons).

Discussion

The aim of cancer treatment is to stimulate apoptosis of cancerous cells. Uncontrolled increase in cell proliferation and dysfunctional apoptosis mediate carcinogenesis.[28] In studies carried with plants, it has been reported that apoptosis was stimulated by cytotoxic effect and the cancer cell death was increased. Turan *et al.* showed in their study that the dimethyl sulfoxideyl extract obtained from the primula vulgaris plant's leafs demonstrated a selective cytotoxic effect on human lung, liver, breast, prostate and colon cancer cell series, compared to normal human fibroblast cells.[29] In another study, it was reported that *P. kurdica* inhibited cell proliferation of HELLA and prostate cancer cell lines with methanol extracts and the ethanol and methanol extracts of *H. calycinum* inhibited cell proliferation in different cancer lines.[30] Herbal medicines such as vinciristine, vinblastine, taxol, camptothecin and podophyllotoxin isolated from plants are used in chemotherapy against cancer.[12]

Many studies have been conducted in different countries trying to uncover the pharmacological activities of *Micromeria* species, especially such as antiseptic, anesthetic, antioxidant, anticholinesterase, antibacterial, antifungal and insecticide. Herken *et al.* reported that *M. congesta* essential oil was found to show high antibacterial activity against 12 bacterial species causing infections in human.[23] Alwan *et al.* reported that the extract of *Micromeria barbata* was particularly useful against multiple resistant pathogens in human infections.[20] Alizadeh and Ranjbaran stated that *Micromeia hedgei* indicated good antimicrobial activity against five important pathogens.[31] In a study conducted in Italy, it was reported that *Micromeria fruticulosa* had antibacterial effect by analyzing the content of the essential oil.[32] The essential oils of *M. persica* in Iran were found to be antimicrobial.[21] This study was the first study examining the cytotoxic and anticancer effect of *M. congesta* essential oil. In our study it was found that *M. congesta* essential oil significantly increased apoptosis (18.00 - 37.00%) on HGC-27 gastric cancer cells compared to control drug. It has been reported that the essential oils obtained from various plants are widely used in traditional medicine and these natural compounds obtained from plants have a potential field of use in the treatment of many diseases, especially cancer.

Micromeria species have been reported to have many pharmacological activities such as antirheumatic diseases, antiseptic, anesthetic, and extracts of some species have antioxidant, anticolinesterase antibacterial, antifungal and insecticide effects. In this study, in which the cytotoxic activity of the essential oil extract of the *M. congesta* plant was also examined, when the results were examined, it was seen that the IC_{50} values in the nine cancer cell series of the extract were in the concentration range of 15.84 - 134.86 µg mL^{-1}. When the values obtained in cancer cell series were compared to the values obtained in normal fibroblast cells, it was understood that the cytotoxic effect of the extract was selective and the most selective cytotoxic effect was obtained in the HGC-27 cell series.

In their study, Koç reported that methanol extract of *P. kurdica* and ethanol and methanol extracts of *H. calycinum* inhibited cell proliferation in HeLa, MCF-7 and prostate cancer cell lines, and in another study, *H. perforatum* species was cytotoxic and antiproliferative.[30,33]

On the other hand, it was reported that the main chemical component of *M. congesta* essential oil, piperitenone oxide (PO), indicated many bioactivities in cells and animals.[34,35] Cancer can be caused by mutations in cell DNA. It is therefore very important that these damaged cells are killed by apoptosis. Apoptotic cell death is controlled genetically. The Bcl-2 family and caspases are the most important genes in the control of apoptosis. Some members of the Bcl-2 family stimulate apoptosis (Proaptoptotik: Bax, Bad, Bid, Bcl-xs), while others inhibit (Anti-apoptotic: Bcl-2, Bcl-xl). In our study, the caspase 3 and caspase 9 gene expressions were examined and 5-FU group was found to be the highest. In the *M. congesta* applied group, the expressions of these apoptosis factor genes were found to be higher than the control group, in accordance with flow cytometry and cell culture results.

In this study, the effects of essential oil extracted from *M. congesta* on the cytotoxic and anticancer activity were studied and essential oil extract was found to have the potential for cancer therapies through advanced drug studies.

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Conflict of interest

There is no conflict of interest to declare.

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