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# Potential therapeutic applications of *Cistus laurifolius* extract: Anti-proliferative, anti-cancer activity on MCF-7, and anti-microbial effects

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ABSTRACT

*Cistus laurifolius* L. is commonly used in Turkish folk medicine for the treatment of ulcers and various types of pain. In this study, the cytotoxic and apoptotic activities of *C. laurifolius* extract on the MCF-7 breast cancer cell line, as well as its antimicrobial activity against eight microorganisms (*Listeria monocytogenes, Enterococcus faecalis, Bacillus subtilis, Staphylococcus aureus, Escherichia coli, S. typhimurium, and Pediococcus acidilactici*) were investigated. Different concentrations (100 $\mu$g/mL, 250 $\mu$g/mL, 500 $\mu$g/mL, and 1000 $\mu$g/mL) of the plant extract were applied for 24, 48, and 72 h, and its effects were determined using MTT, BrdU, and *in situ* apoptosis detection assays on human breast cancer cell MCF-7. Prolonged high-dose application of *C. laurifolius* extract led to decreased cell viability, inhibition of DNA synthesis, and induction of apoptosis. Its potential to induce cell death, which is the most desirable pathway for cancer treatment, is noteworthy alongside its cytotoxic activity. The antimicrobial effect of *C. laurifolius* extract varied depending on the Gram reaction of the microorganisms, with the extract being more effective against Gram-positive bacteria than Gram-negative bacteria. In conclusion, the *C. laurifolius* extract exhibits cytotoxic, anticancer, and antimicrobial properties.



## 1. Introduction

Cistus L. (Cistaceae) is commonly found plant across the Mediterranean region. Traditional medicinal practices utilize its flowers, leaves, and aerial parts to address conditions such as the common cold, peptic ulcers, wounds, and a range of inflammatory disorders (Guzelmeric et al., 2023). It is known as 'laden' or 'karahan' in Turkish and 'rock rose' or 'sun rose' in English (Sadhu et al., 2006; Akkol et al., 2012). Extracts of Cistus species have been reported to possess anti-ulcer, anti-spasmodic, antimicrobial, antiviral, antioxidant, and cytotoxic properties (Guvenc et al., 2005; Gurbuz et al., 2015; Mahmoudi et al., 2016). Methanol extraction from the leaves and small branches of *Cistus laurifolius* L. (Sadhu et al., 2006) and aqueous ethanol extraction from the leaves (ellagic acid, apigenin, quercetin, and kaempferol) of *C. laurifolius* (Orhan et al., 2013) have antioxidant properties. It has been determined that the water extract of *C. laurifolius* has three main components: gallic acid, quercetin, and apigenin, which have anti-inflammatory activity (Pekacar et al., 2024). In another study, when the water extract of *C. laurifolius* was examined for its phenolic content, it was determined that rutin and gallic acid were the main components. The extract contains protocatechuic acid, 4-hydroxybenzoic acid, caffeic acid, syringic acid, epicatechin, p-coumaric acid, ferulic acid, rutin, resveratrol, daidzein, luteolin, trans-cinnamic acid, hesperetin, chrysin, pinocembrin, and caffeic acid phenyl ester, excluding catechin and myricetin. Extracts with this content have been reported to possess antioxidant capacity (Hacioglu et al., 2021). Aqueous extracts of *C. ladanifer* and *C. populifolius*, both belonging to the Cistus L. family, were investigated for their cytotoxic effects on M220 pancreatic cancer cells, MCF7/HER2, and JIMT-1 breast cancer cells. It was determined that, in addition to their antimicrobial properties, they inhibited the proliferation of cancer cells (Barrajon-Catalan et al., 2010). Hexane extracts of *Cistus libanotis, Cistus villosus*, and *Cistus monspeliensis* also exhibited cytotoxic activity against three cancer cell lines, including murine monocyte/macrophages J774.A1, the human melanoma cell line A-375, and the human breast cancer cell line MCF7 (Jemia et al., 2013). The cytotoxic properties of hexane, methylene chloride, and ethanol extracts of *C. laurifolius* were also investigated in the A549, DU-145, PNT-1A, MDA-MB231, CRL-4010, and HCT-116 cell lines. It has been determined that different extracts cause different cytotoxicities. It has been suggested that *C. laurifolius* L. may have the potential to be used as an anticancer and antioxidant agent (Budak et al., 2022).

There are few studies on the antimicrobial properties of Cistus spp. (Tomas-Menor et al., 2013; Morales-Soto et al., 2015) *C. ladanifer*

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and *C. albidus* have been studied more extensively than others (Morales-Soto et al., 2015). However, information on the antimicrobial properties of *C. laurifolius* is limited. Only one study focused on the anti-*Helicobacter pylori* activity of *C. laurifolius* to demonstrate the anti-ulcerogenic activity of plant flavonoids (Ustun et al., 2006). Moreover, no studies have reported the anti-microbial activity of *C. laurifolius* on a variety of microorganisms that cause several diseases and are important in food microbiology

The aim of this study was to investigate whether the extract obtained from the leaves of *C. laurifolius* induced anti-cancer activity in human breast cancer cells (MCF-7), and to determine its potential antimicrobial activity.

## 2. Material and methods

### 2.1. Plant extraction method and cell culture

*C. laurifolius* were collected from Eskişehir. It was identified by Professor Ersin Yucel. Leaves of the plant material were used for the experiments and dried at room temperature. Powdered *C. laurifolius* (40 g) was extracted in 800 mL distilled water at 65 °C for 1 h. After cooling, the extract was filtered through filter paper, concentrated using a rotary evaporator, and stored at 4 °C (Hacioglu et al., 2021). For the *in vitro* assay, the extract was resuspended in medium containing DMSO (at a final concentration of 0.1%) and filtered through 0.22 $\mu$m filters.

The human breast cancer cell line MCF-7 was maintained in MEM supplemented with 10 % FBS, sodium pyruvate (100 mM), bovine insulin (100 IU/mL), and sodium bicarbonate (7.5 mg/mL) and incubated at 37 °C in a humidified atmosphere of 5 % $CO_2$. A stock solution of *C. laurifolius* extract was diluted to the following concentrations: 100, 250, 500, and 1000 $\mu$g/mL and applied to cells for 24, 48, and 72 h. DMSO was used as the negative control in all experiments.

### 2.2. Cell viability analysis

To determine the cell viability effects of the extract, a 3-(4,5-Dimethylthiazol-2-yl)−2,5-Diphenyltetrazolium Bromide) assay was performed. After the incubation period, the medium with extract concentrations was discarded, and 100 $\mu$L fresh medium containing 0.5 mg/mL MTT (Sigma) dissolved in phosphate-buffered saline (PBS) was added to each well. The plates were then incubated for 3 h at 37 °C. After this incubation, blue formazan crystals were dissolved in DMSO and the results were quantified by measuring the absorbance at 570 nm using an ELx808 Absorbance Microplate Reader (Bio-Tek, USA). All experiments were performed in eight parallel with three replications.

### 2.3. DNA synthesis assay with BrdU incorporation

BrdU (5-bromo-2′-deoxiuridine) is a nucleoside analog of the DNA precursor thymidine. In proliferating cells, DNA is replicated before cell division. If BrdU, as a synthetic nucleotide, is added to the cell culture, proliferating cells incorporate it into their DNA (Vega-Avila and Pugsley, 2011). DNA synthesis of cells was assessed by *in vitro* using a BrdU proliferation ELISA kit (Roche) according to the manufacturer's instructions. Ten $\mu$L of BrdU labeling solution was added to each 100 $\mu$L of well and plates were incubated again for 2 h at 37 °C. Labeling medium was removed and then 200 $\mu$L of FixDenat solution were added and wells were incubated for 30 min at room temperature. After removing of FixDenat solution, wells were incubated with 100 $\mu$L anti-BrdU-POD working solution for 90 min at room temperature. Wells were rinsed three times with 200 $\mu$L/well of Washing Solution. After removing the Washing Solution, 100 $\mu$L/well of substrate solution was added, and the wells were incubated for 30 min. After adding stop solution, absorbance was measured at 450 nm.

### 2.4. Determination of apoptosis by TUNEL assay

Apoptotic cells were determined by TUNEL method (Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling). In situ apoptosis detection kit (MK500, TAKARA) was used to measure apoptotic index. During apoptosis process, 3′-OH ends of DNA fragments are generated, terminal deoxynucleotidyl transferase is used to label these ends. Cells were fixed with 4 % paraformaldehyde / PBS solution (pH7.4) at room temperature for 20 min. After fixation, endogenous peroxidase was inactivated with methanol containing 0.3 % $H_2O_2$ at room temperature for 20 min. After washing steps with PBS, 100 $\mu$L of Permeabilization Buffer was applied on ice for 5 min. Five $\mu$L TdT Enzyme and 45 $\mu$L Labeling Safe Buffer were mixed and this labeling reaction mixture was applied and slides were incubated in a 37 °C humidified chamber for 90 min. Reaction was terminated by washing with PBS. Seventy five $\mu$L Anti-FITC HRP Conjugate was applied and slides were reincubated at 37 °C for 30 min. After incubation, slides were colored by applying DAB for 10 min. Cells were stained with 3 % methyl green. Apoptotic cells were detected with a light microscope. The apoptotic index was determined by counting at least 500 nuclei subdivided in 5 fields chosen randomly. Apoptotic index was calculated as the number of apoptotic cells, expressed as percentage of total number of cells counted in each case.

### 2.5. Anti-microbial activity

#### 2.5.1. Preparation of bacterial strains

Five Gram-positive bacteria (*Listeria monocytogenes* Scott A, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6037, *Staphylococcus aureus* 6538P and *Pediococcus acidilactici* ATCC 8042) and three Gram-negative bacteria (*Escherichia coli* O157:H7 ATCC 43895, *Salmonella* Typhimurium NRRLB4420 and *E. coli* ATCC 1103) were tested for evaluating the antimicrobial activity of *C. laurifolius* extract. All microorganisms were obtained from Food Microbiology Laboratory, Food Engineering Department, Ege University, Turkey. Stock cultures were activated in Mueller-Hinton Broth (MHB, pH 7.3 ± 0.2, Oxoid, CM405) at 37 °C for 24 h. The optimized bacterial cultures (DEN-1 Mc Farland Densitometer, Grant-bio), equivalent to 0.5 McFarland turbidity standard, were used in the analyses.

#### 2.5.2. Preparation of extract

*C. laurifolius* extract was re-dissolved in sterile distilled water and the extract solution (40 mg/mL) was filter-sterilized (Minisart Syringe Filter, Cellulose Asetate, Sartorious Stedim Biotech, SM16534K).

#### 2.5.3. Screening for the antimicrobial properties by using agar disc diffusion assay

The potential antimicrobial activity of the prepared extract solution was determined by agar disc diffusion from Deng et al. (2014) with some modifications. In the application of disk diffusion assay, bacterial cultures (at $10^6$ CFU/g) were spread on Muller Hilton Agar (MHA, pH 7.3 ± 0.2, Oxoid, CM337) and allowed to dry for 20 min. Sterilized blank disks (6 mm diameter, Oxoid, CT0998B) were dipped into the prepared extract solution (20 $\mu$l/disk) and allowed to dry in the laminar flow. Then prepared disks were placed on the seeded plates with negative control (sterile water) and positive control (ampicillin (10 $\mu$g/disk, Oxoid, CT0003B) and gentamicin (10 $\mu$g/disk, Oxoid, CT0024B)) disks. The inhibition zones were measured after 24 h of incubation at 37 °C.

#### 2.5.4. Determination of minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC)

The MIC of the extract was determined using a 96-well microtiter plate method according to the modified protocol described by Deng et al. (2014). To determine the minimum inhibition concentration of

*C. laurifolius* extract, 96-well "U" type sterile microplates were used. In the first stage, 100 $\mu$L of Mueller Hinton Broth (MHB) medium was added to each well. In the second stage, 100 $\mu$L of the extracts at 40 mg/mL concentration was added to the wells in the first row of the plate, and then 100 $\mu$L of the 200 $\mu$L extract medium mixture found in the first row was drawn and transferred to the next well, and 100 $\mu$L of the mixture found in the wells of the second row was added to the third row of wells, and this dilution process was applied in a similar manner from the last row of the plate to the two previous rows, the last 100 $\mu$L volumes drawn were thrown out, and thus the dilution of the extract was carried out between wells 1–10 (20 mg/mL - 0, 0390 mg/mL) has been completed. Twenty four hour liquid cultures adjusted to a 0.5 McFarland turbidity standard (approximately $10^8$ cfu/mL) were diluted ten fold and added to all wells in volumes of 10 $\mu$L after adjusting each to approximately $10^7$ cfu/mL. Only culture (10 $\mu$L) was added to the eleventh row wells for positive control. Apart from this, medium (100 $\mu$L) and extract solution (100 $\mu$L) were added to the wells in the twelfth row for negative control. Thus, in each microplate, the extract concentration is 18.181 mg/mL, 9.090 mg/mL, 4.545 mg/mL, 2.272 mg/mL, 1.136 mg/mL, 0.568 mg/mL, 0.284 mg/mL, 0.142 mg/mL, 0.071 mg/mL. Wells with 0.035 mg/mL were obtained. Wells containing only MHB and the test cultures were used as control samples. After incubating the plates at 37 °C for 18 h, 20 $\mu$L of 0.5 % (w/v) 2,3,5-triphenyl tetrazolium chloride (TTC, Merck, 108,380, Germany) aqueous solution was added into the wells and the color change of the wells were interpreted after 30 min at 37 °C. The lowest concentration of the extract required to inhibit visible growth of the test culture (no color formation) was selected as the MIC value. To determine the minimum bactericidal concentration of the extract, inoculum was transferred from the well selected as MIC on MHA and plates incubated at 37 °C for 24 h were checked for colony formation.

### 2.6. Statistical analyses

Experiments were performed in triplicate. Values are expressed as means±standard deviations (SDs). Statistical analysis was done by using SPSS 16.0 software. One-way analysis of variance (ANOVA) followed by the Tukey test was used to determine the statistical significance between groups. a $p < 0.05$ was considered to be statistically significant difference.

## 3. Results

### 3.1. Cell viability analysis by MTT assay

Fig. 1 shows percentage of cell viability measured by the MTT assay. As seen in Fig. 1, one hundred $\mu$g//mL of extract at 48 h was not different from the control group at the same time point. Similarly, the extract applied at concentration of 250 $\mu$g//mL was not different from the same concentration at 24 and 72 h and was not statistically different from the control group. Only the extract applied at concentration of 500 $\mu$g//mL was significantly different at 48 h compared to the other two time points (24 and 72 h), but it is not different from the concentration of 250 $\mu$g//mL. It is clear from the results that MCF-7 cell exposure to 1000 $\mu$g/mL Cistus extracts for 72 h led to significant inhibition of cell viability, but other concentrations did not significantly inhibit cell viability.

### 3.2. DNA *synthesis* assay

Fig. 2 shows the DNA synthesis with BrdU incorporation in the MCF-7 breast cancer cell line after 24, 48, and 72 h of exposure to different concentrations of *C. laurifolius* extract. It was determined that 1000, 500, and 250 $\mu$g/mL caused statistically significant inhibition of DNA synthesis when applied for 24, 48 and 72 h ($p < 0.05$).

One hundred $\mu$g/mL concentration of extract also had inhibitor effect on DNA synthesis but this effect was not drastic as the other concentrations ($p < 0.05$) (Fig. 2).

### 3.3. Apoptosis determination by TUNEL assay

In order to determine apoptotic index, apoptotic cells were counted in each flask that was photographed. In each flask, 5 different areas were evaluated, and approximately 500 cells were counted. Apoptotic index was calculated following formula:

$$\text{Apoptotic index} = \text{apoptotic cells}/\text{total cells} \times 100$$

Plant extractions at 100, 250 and 500 $\mu$g/mL had no effect to result in apoptosis when compared to control. On the other hand, 1000 $\mu$g/mL induce apoptotic cell formation following all hours of exposure as seen in Fig. 3 ($p < 0.05$).

In the process of apoptosis, intracellular endonucleases trigger the fragmentation of DNA. The In Situ Apoptosis Detection Kit is specifically created to histochemically identify fragmented DNA through TUNEL (TdT-mediated dUTP Nick End Labeling). Using this technique, fluorescein-labeled nucleotides are integrated onto the 3′ ends of DNA fragments *in situ*, enabling the precise localization and detection of individual apoptotic cells. As seen in Fig. 4, the highest concentration, 1000 $\mu$g/mL, induced apoptotic cells. White arrows show apoptotic cells in photos.

### 3.4. Anti-microbial activity

*Cistus laurifolius* extract was screened for the anti-microbial activity against Gram-positive bacteria (*Listeria monocytogenes* Scott A, *Enterococcus faecalis* ATCC 29212, *Bacillus subtilis* ATCC 6037, *Staphylococcus aureus* 6538P and *Pediococcus acidilactici* ATCC 8042) and Gram-negative bacteria (*E. coli* O157:H7 ATCC 43895, *S.* Typhimurium NRRLB4420 and *E. coli* ATCC 1103). The inhibition zones (mm) of the extract on test cultures were given in Table 1.

The preliminary screening results indicated that the anti-bacterial activity of *C. laurifolius* extract is closely related with the microorganisms. The extract showed inhibitory effect against all microorganisms tested, except *E. coli* (Table 1). For all cultures screened in the agar diffusion assay, *S. aureus* demonstrated the highest zone of inhibition and it was followed by *B. subtilis*.

The minimum inhibitory concentration (MIC) of *C. laurifolius* extract was determined on eight microorganisms (*Listeria monocytogenes Scott A, Enterococcus faecalis ATCC 29212, Bacillus subtilis ATCC 6037, Staphylococcus aureus 6538P, E. coli O157:H7 ATCC 43895, S.* Typhimurium *NRRLB4420, E. coli* ATCC 1103, *Pediococcus acidilactici* ATCC 8042) using a 96-well microtiter plate method, containing the final concentrations of the extract (18.181, 9.090, 4.545, 2.272, 1.136, 0.568, 0.284, 0.142, 0.071 and 0.035 mg/mL). The MIC values of the extract are given in Table 2. The results, reported in mg/mL, indicated that except *E. coli*, all the bacterial strains, both Gram-positive and Gram-negative, were inhibited by the extract. The MIC values were ranged from 2.272 to 18.181 mg/mL. The most sensitive bacteria to the extract were found as *S. aureus, B. subtilis* and *E. faecalis*.

The MBC of the extract were given in Table 2. The results showed that MBC values were higher than MIC values for L. *monocytogenes, E. faecalis, B. subtilis* and *S. aureus*, while the values were equal for *E. coli* O157:H7 and *P. acidilactici*. Moreover, no bactericidal effect was observed for *S.* Typhimurium and *E. coli* at concentrations used in the study.

## 4. Discussion

Cancer is a complex disease, and approaches to its treatment are inevitably intricate. Although conventional treatment approaches involve chemotherapy, surgery, and radiotherapy, combinations of

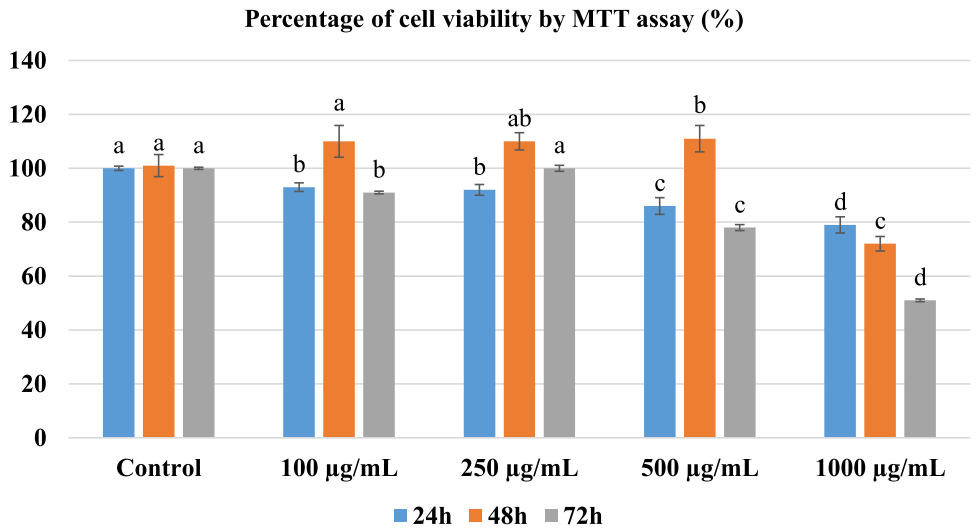


**Fig. 1.** Cell viability in MCF-7 breast cancer cell line when exposed to different concentrations of *Cistus laurifolius* for 24, 48 and 72 h. Groups at each hour with different letters are statistically different ($p < 0.05$), groups marked with the same letter are not statistically different.

these are preferred for full recovery. Unfortunately, owing to the side effects of these methods, exploring alternative approaches is necessary, and solutions derived from nature hold their own potential (Roy et al., 2022). In this study, we determined that the highest concentration of *C. laurifolius* extract exhibited cytotoxic activity, suppressed DNA synthesis, and induced apoptosis at 72 h.

MTT is used as a metabolic cell proliferation assay and is commonly employed to screen the cytotoxic effects of drug/extracts (Adan et al., 2016). It is clear from the results that MCF-7 cell exposure to 1000 $\mu$g/mL Cistus extracts for 72 h led to significant inhibition of cell viability, but other concentrations did not significantly inhibit cell viability. As shown in Fig. 1, the extract did not exhibit cytotoxic effects, except at concentration of 1000 $\mu$g/mL. In cytotoxicity studies, for a drug to be considered cytotoxic, the cell viability should not exceed 70 % (ISO 10,993−5:2009; Biological evaluation of medical devices part-5: Tests for *in vitro* cytotoxicity).Therefore, the remaining concentrations (100, 250 and 500 $\mu$g/mL) were not toxic to the metabolic activity of the cells. The doubling time of MCF-7 cells is also approximately 38 h (Amalia et al., 2020; Daddiouaissa et al., 2019). At 48 h, it was possible for MCF-7 cells to proliferate and showed different results in the MTT test because they were not affected by the low concenrations of the extract. In our study, we applied the BrdU test to assess cell proliferation in the S phase. DNA synthesis assays with BrdU incorporation measure cell proliferation during the synthesis (S) phase of the cell cycle. Therefore, precise cell proliferation measurements are needed to study cell cycle regulation and cell division (Adan et al., 2016; Reischmann et al., 2015). The BrdU assay is more sensitive than the MTT assay. These differences can be attributed to the distinct focus on mitochondrial activity and DNA synthesis (Reischmann et al. 2015). As a result, MTT is a measure of mitochondrial activity that is proportional to total cell number. BrdU incorporation is a measure of DNA synthesis rate reflecting the rate of proliferation, not the number of cells in the well. Therefore, It is possible for MTT and BrdU tests to yield different results (Chan et al., 2013).

Cistus spp. extracts had an anti-proliferative effect on OVCAR and MCF-7 cells at 5 mg/mL of extract (El Euch et al., 2015), and *C. laurifolius* extract at 1000 $\mu$g/mL, 500 $\mu$g/mL, 250 $\mu$g/mL $\mu$g/mL also exerted cytotoxic activity on human cervical adenocarcinoma cells (Hep2C), human muscle rhabdomyosarcoma cells (RD), and mouse fibrosarcoma cells (Wehi 164) (Soydam Aydın and Yücel, 2021). Guzelmeric et al. (2023) also showed that 1 mg/mL *C. laurifolius*

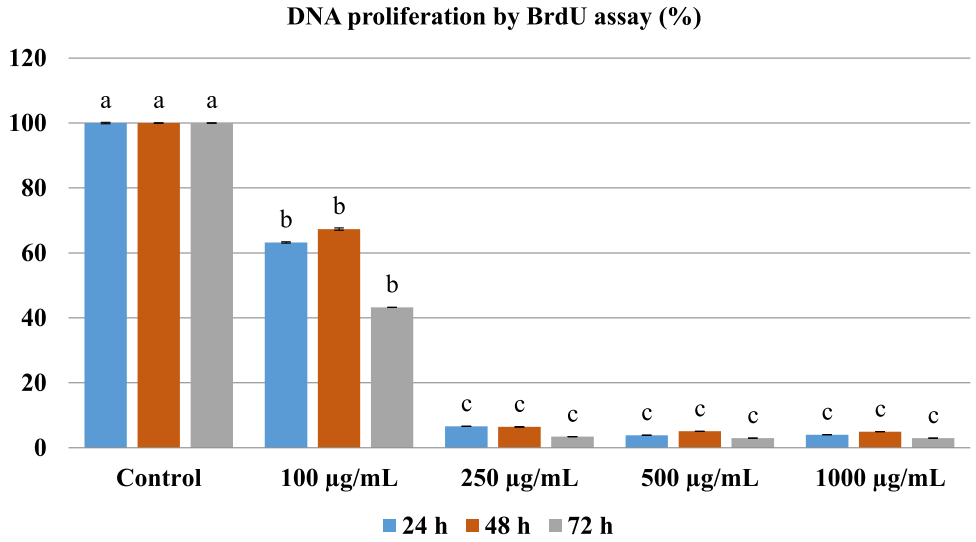


**Fig. 2.** DNA synthesis in cells that are exposed to plant extract concentrations for 24, 48 and 72 h. Groups at each incubation time with different letters are statistically different ($p < 0.05$), groups marked with the same letter are not statistically different.

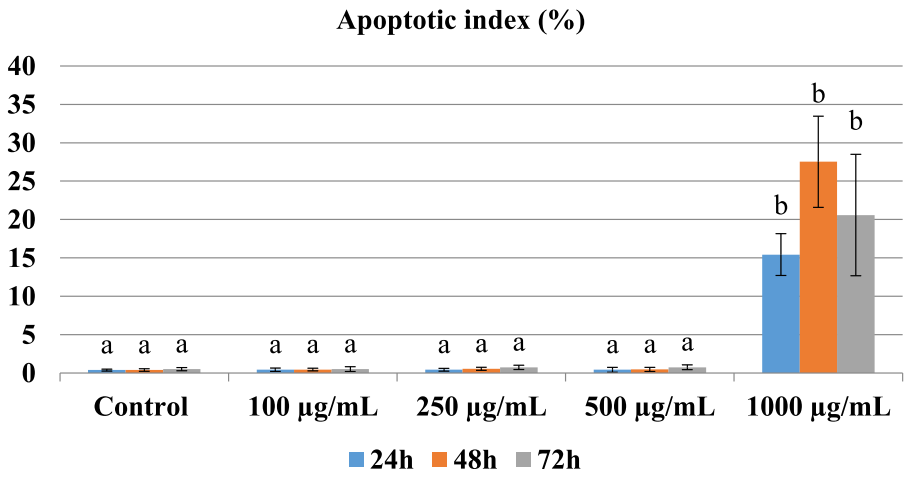


**Fig. 3.** Apoptotic index for 24, 48 and 72 h. Groups at each hour with different letters are statistically different ($p < 0.05$), groups marked with the same letter are not statistically different.

extract exerted anticancer activity against pancreatic cell lines MIA PaCA-2 in both 2D and 3D models. In another study, extracts of five Cistus species (*C. laurifolius, C. albicans, C. salviifolius, C. parviflorus, and C. albicans*) were analyzed in terms of cytotoxicity in MCF-7 cells, and it was found that *C. laurifolius* had no cytotoxicity in these cell lines (Onal et al., 2023). The studies conducted on *C. laurifolius* mostly aimed to determine whether it exhibited cytotoxicity using MTT. Studies on other Cistus spp. have shown their potential anti-cancer and anti-proliferative effects (Barrajón-Catalán et al., 2010; Vitali et al., 2011; Skoric´ et al., 2012). In the present study, the cytotoxicity of *C. laurifolius* extract on MCF-7 cells was evaluated both through MTT analysis and by assessing its effects on DNA synthesis and apoptosis. The highest concentration of the extract induced apoptosis and inhibited DNA synthesis. The cytotoxic effects also vary depending on the preparation method of the plant extract (Budak et al., 2022). However, Cistus spp. contains many secondary metabolites that can be toxic when used in large amounts. Therefore, special attention should be paid to the amounts consumed (Papaefthimiou et al. 2014). It was confirmed that *C. laurifolius* was characterized by antioxidant properties. In the extract from the leaves and small branches of *C. laurifolius*, the presence of 16 bioactive compounds was determined by 1 H and 13C NMR techniques and EI-MS mass spectrometry. The following compounds from *C. laurifolius* have been shown to capture free radicals: 3–0-methyl quercetin (1), 3,7–0-dimethyl quercetin (2), ellagic acid (8), quercetin 3–0-$\alpha$-rhamnoside (10),1-(4-hydroxy-3-methoxyphenyl)–2-[4-(3-$\alpha$-L-rhamnopyranoxypropyl)–2-methoxyphenoxy]–1,3-propanediol (12), olivil 9-O-$\beta$-D-xyloside (13), berchemol 9-O-rhamnoside (14), and (7S,8R)-dihydrodehydrodiconiferyl alcohol 9′-O-$\alpha$-L-rhamnoside (major isomer) (16) (Stepien et al., 2018). All of these components obtained from water extraction may contribute to its cytotoxic activity, inhibition of DNA synthesis, and induction of apoptosis.

This study also demonstrated the ability of the *C. laurifolius* extract to inhibit various microorganisms, including pathogens. Of the

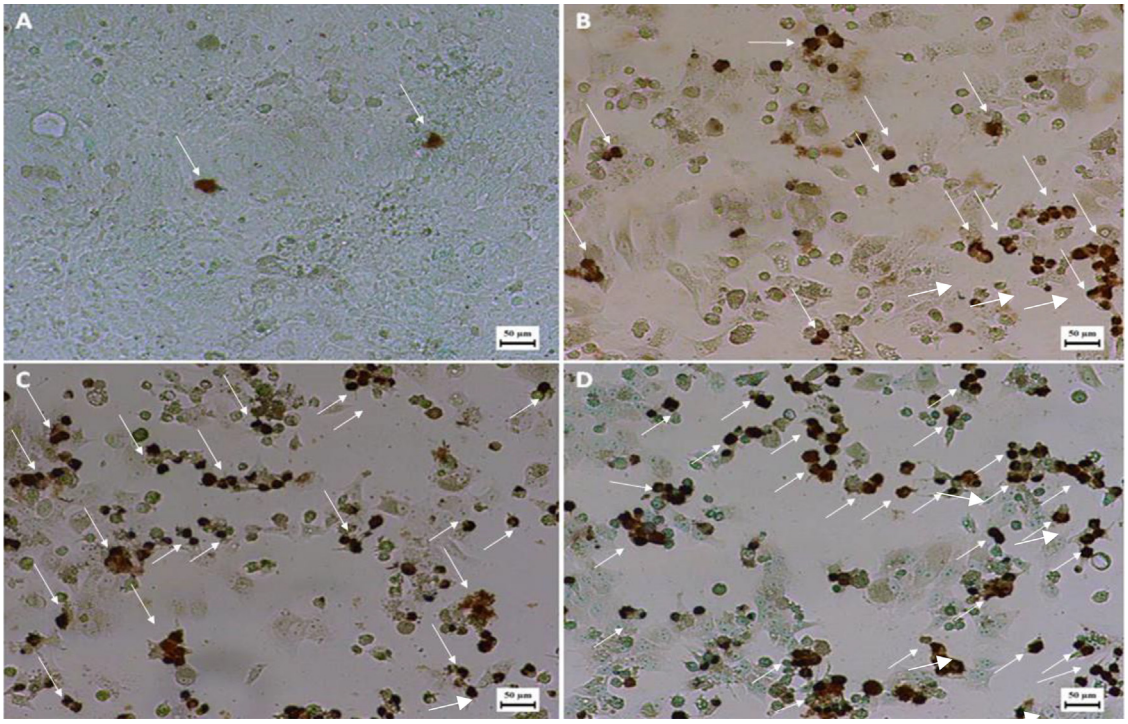


**Fig. 4.** Control cells (A) and red-brown apoptotic cells when 1000 $\mu$g/mL was applied for 24 h (B), 48 h (C) and 72 h (D).

**Table 1**
The inhibition zones of *Cistus laurifolius* extract on test cultures.

| Microorganisms | Inhibition zone (mm) | | | |
|---|---|---|---|---|
| | *C. laurifolius* extract | Distilled water (Negative control) | Gentamicin (Positive control) | Ampicillin (Positive control) |
| *Listeria monocytogenes* | 7 | – | 28 | 30 |
| *Enterococcus faecalis* | 7 | – | 14 | 28 |
| *Bacillus subtilis* | 8 | – | 34 | 34 |
| *Staphylococcus aureus* | 10 | – | 28 | 48 |
| *E. coli* O157:H7 | 7 | – | 22 | 12 |
| *S.* Typhimurium | 7 | – | 24 | 24 |
| *E. coli* | – | – | 24 | 24 |
| *Pediococcus acidilactici* | 7 | – | 38 | 38 |

(-): No inhibition.

cultures analyzed, *S. aureus, B. subtilis* and *E. faecalis* were the most susceptible to *C. laurifolius* extract, with the lowest MICs/MBCs. *C. laurifolius* extract did not demonstrate any inhibition zone or MIC on *E. coli* and not demonstrated MBC on *S. Typhimurium* and *E. coli*, unlike the other cultures tested. All other microorganisms tested were very sensitive to the extract, with MICs ranging between 2.272–18.181 mg/mL, depending on the test culture. A study by Barrajón-Catalán et al. (2010), *C. ladanifer* and *C. populifolius* extracts inhibited *S. aureus* (MIC 154 $\mu$g/mL) and *E. coli* (MIC 123 $\mu$g/mL), respectively. Ustun et al. (2006) investigated antimicrobial activity of chloroform fractions of the leaf extract of *C. laurifolius* against *Helicobacter pylori* and found that quercetin 3-methyl ether has the highest inhibitory effect on *H. pylori* (MIC 3.9 $\mu$g/mL). The results obtained from these studies showed differences depending on the Cistus spp., extraction method, and target microorganism used. The volatile fraction, which contains terpenes and polyphenolic compounds, is the main component responsible for antimicrobial activity of Cistus spp. (Barrajón-Catalán et fraction, which contains terpenes and a polyphenolic fraction (Tomas-Menor et al., 2013; Morales-Soto et al., 2015; Barrajón-Catalán et al., 2010). *C. laurifolius* extract is a rich source of phenolic compounds that are more soluble in ethanol than in water (Akkol et al., 2012; Orhan et al., 2013). In our study, a water extract was used as the test material. Hence, some bioactive compounds may not be extracted from the plant, which could explain why high concentrations of *C. laurifolius* are required for significant cytotoxic and antimicrobial effects. The results of the study indicated that *C. laurifolius* extract showed higher inhibitory effect on Gram-positive bacteria (*Listeria monocytogenes, Enterococcus faecalis, Bacillus subtilis, Staphylococcus aureus* and *Pediococcus acidilactici*) than Gram-negative bacteria (*E. coli* O157:H7, *S. Typhimurium* and *E. coli*) that might be associated with the differences in cell surface structures of them. These findings suggest that the mechanism of inhibition is mediated by membrane interactions. Similarly, Demetzos et al. (2002) reported that the essential oil of *C. salviifolius* has the highest inhibitory effect on gram-positive bacteria.

**Table 2**
MIC and MBC of *Cistus laurifolius* extract on eight microorganisms.

| Microorganisms | MIC Values (mg/mL) | MBC Values (mg/mL) |
|---|---|---|
| *Listeria monocytogenes* | 4.545 | 9.090 |
| *Enterococcus faecalis* | 2.272 | 4.545 |
| *Bacillus subtilis* | 2.272 | 4.545 |
| *Staphylococcus aureus* | 2.272 | 4.545 |
| *E. coli* O157:H7 | 18.181 | 18.181 |
| *S.* Typhimurium | 18.181 | -[b] |
| *E. coli* | -[a] | -[b] |
| *Pediococcus acidilactici* | 9.090 | 9.090 |

[a]: No inhibitive effect for *C. laurifolius* extract concentrations used in the study.
[b]: No bactericidal effect for *C. laurifolius* extract concentrations used in the study.

In the present study, the effects of *C. laurifolius* extract as a traditional medicinal plant were evaluated using *in vitro* anti-cancer and anti-microbial assays. High concentration of *C. laurifolius* extract had cytotoxic and apoptotic effects. These results can be applied in the fields of preventive medicine and food safety. However, further *in vitro* and *in vivo* studies are needed to elucidate the molecular and biochemical mechanisms of *C. laurifolius*.

## Declaration of competing interest

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## CRediT authorship contribution statement

**Ersin Yücel:** Methodology, Supervision. **Ayşe Ak:** Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Writing – original draft, Writing – review & editing. **İlkin Yücel Şengün:** Investigation, Writing – original draft, Writing – review & editing. **Hatice Genç:** Investigation, Writing – original draft. **Tansu Koparal:** Investigation, Writing – original draft. **Hülya Sivas:** Investigation.

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