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Investigation of Antioxidant, Antimicrobial and Cytotoxic Activity of *Cydonia oblonga* Leaf on Breast Cancer (MCF-7) and Liver Cancer (HepG2) Cell Lines

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Abstract: Cancer, one of the most common pathologies in the world, has been reducing the standard of living of many people for centuries and can cause death. Many medicinal plants are widely used in cancer treatment due to their ability to improve disease symptoms and low side effects. *Cydonia oblonga* (*C. oblonga*) leaf, which is among the medicinal plants and has important health properties, is a type of plant commonly known as quince leaf. Although it originates from Asia, it is cultivated in many countries today. In this study, antioxidant, antimicrobial and cytotoxic activities of *C. oblonga* leaves on MCF-7 and HepG2 cell lines were investigated. As a method, the collected *C. oblonga* leaves were extracted in methanol and hexane solvents. The extracts were tested on MCF-7 and HepG2 cell lines using MTT Assay method for cytotoxic activity, DPPH method for antioxidant activity and well agar method for antimicrobial effects of the extract on five different microorganisms. As a results, although *C. oblonga* leaf in both cell lines hexane extract exhibited stronger cytotoxic activity than methanol extract, methanol extract was found to have stronger antioxidant effect than hexane extract. The extracts applied to microorganisms showed the best zone diameter effect on *E. coli* and the lowest effect on *B. megaterium*. In addition, it was observed that *C. oblonga* leaf has stronger antimicrobial effects than anticancer and antioxidant activity.

Key words: *Cydonia oblonga*, cytotoxic activity, antioxidant activity, antimicrobial activity.

Cydonia oblonga Yaprığının Meme Kanseri (MCF-7) ve Karaciğer Kanseri (HepG2) Hücre Hatları Üzerindeki Antioksidan, Antimikrobiyal ve Sitotoksik Aktivitesinin Araştırılması

Öz: Dünyadaki en yaygın patolojilerden biri olan kanser, yüzyıllardır birçok insanın yaşam standardını düşürmekte ve ölüme neden olabilmektedir. Birçok şifalı bitki, hastalık semptomlarını iyileştirme yetenekleri ve düşük yan etkileri nedeniyle kanser tedavisinde yaygın olarak kullanılmaktadır. Şifalı bitkiler arasında yer alan ve sağlık açısından önemli özelliklere sahip olan *Cydonia oblonga* (*C. oblonga*) yaprağı, halk arasında ayva yaprağı olarak bilinen bir bitki türüdür. Asya kökenli olmasına rağmen günümüzde birçok ülkede yetiştirilmektedir. Bu çalışmada, *C. oblonga* yapraklarının MCF-7 ve HepG2 hücre hatları üzerindeki antioksidan, antimikrobiyal ve sitotoksik aktiviteleri araştırılmıştır. Yöntem olarak, toplanan *C. oblonga* yaprakları metanol ve hekzan çözücülerinde ekstrakte edilmiştir. Ekstraktlar MCF-7 ve HepG2 hücre hatları üzerinde sitotoksik aktivite için MTT Assay yöntemi, antioksidan aktivite için DPPH yöntemi ve ekstraktın beş farklı mikroorganizma üzerindeki antimikrobiyal etkileri için well agar yöntemi kullanılarak test edilmiştir. Sonuç olarak, *C. oblonga* yaprağı her iki hücre hattında da hekzan özütü metanol özütüne göre daha güçlü sitotoksik aktivite sergilemesine rağmen, metanol özütü hekzan özütüne göre daha güçlü antioksidan etkiye sahip bulunmuştur. Mikroorganizmalara uygulanan ekstraktlar en iyi zon çapı etkisini *E. coli* üzerinde, en düşük etkiyi ise *B. megaterium* üzerinde göstermiştir. Ayrıca, *C. oblonga* yaprağının antikanser ve antioksidan aktiviteden daha güçlü antimikrobiyal etkilere sahip olduğu gözlemlenmiştir.

Anahtar kelimeler: *Cydonia oblonga*, sitotoksik aktivite, antioksidan aktivite, antimikrobiyal aktivite.

1. Introduction

Cancer is one of the most alarming diseases of the 20th century and continues to spread in the 21st century due to its persistence and increasing incidence. This is a cause for concern as one in four people have a lifetime risk of cancer [1]. Human breast cancer (MCF-7) is one of the most common cancers and has become one of the most common causes of cancer death in women [2]. Human liver cancer, of which 75-90% are hepatocellular carcinomas (HepG2), it is the third leading cause of cancer-related deaths in men and the sixth leading cause in women [3]. The progression of cancer, which is so widespread and deadly, is a multi stage process in which cells

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must overcome various obstacles before they become fully developed tumors. Although significant advances have been made in recent years in treatments to stop cancer growth, many patients have to fight against metastasis and tumor growth due to chemoresistance with challenging radiotherapy and chemotherapy. Researchers are therefore exploring new methods to better understand the behavior of cancer cells and develop more effective treatments. Due to some serious side effects and the medical and economic problems associated with the use of synthetic drugs for cancer treatment, herbal therapies have become popular. Herbal medicines are considered a safe, non toxic and easily accessible source of compounds that can treat cancer. Plants are believed to neutralize the effects of diseases on the body because of the active compounds they contain [4]. It has been suggested that natural antioxidants reduce the toxic effects of anticancer treatment processes and that the use of nutraceuticals may improve the treatment more, with the idea that it may help patients undergoing cancer treatment in this process [5]. Accordingly, over the years, the determination of the cytotoxic properties of natural products has gained great importance [6-9]. Quince leaf, known as *C. oblonga*, is a plant belonging to the Rosaceae family and is native to the Mediterranean region and Central Asia. This plant leaf has a long history of ethnobotanical and medicinal use [10]. The leaves of the *C. oblonga* are egg shaped or broadly elliptical, dark green and toothless on the margins, but these young leaves are petiolate. It can generally be grown in all regions in the temperate zone. Known for its antidiabetic, antimicrobial, antioxidant and UV (ultraviolet)-protective abilities, the biological phytochemicals in the leaves of this plant are also known to be a promising source of natural healing [11]. *C. oblonga* leaf is a plant recognized as an active ingredient with many characteristic properties, mainly antioxidant [12]. When the leaves of the plant are consumed by boiling and straining, it is known to have calming, antipyretic, antidiarrheal and antitussive properties [13-16]. In addition to having an important place in folk medicine, it is also considered as a good and economical source of dye [17]. *C. oblonga* leaf and fruit extracts have been found to exhibit antiproliferative activities, suggesting that this plant leaf may inhibit or reduce the growth of cancer cells [18]. Plants with antimicrobial activity are used as preservatives in foods, for medical purposes, and for their effects against parasites and microorganisms [19].

In the light of this information, the main aim of this study is to extract the leaves of *C. oblonga* plant as shown in the project flow chart in Figure 1 and to show its cytotoxic activity on MCF-7, HepG2 cell lines and to investigate its antimicrobial and antioxidant effects at different concentrations and to provide the molecular mechanisms of these activities to the literature.

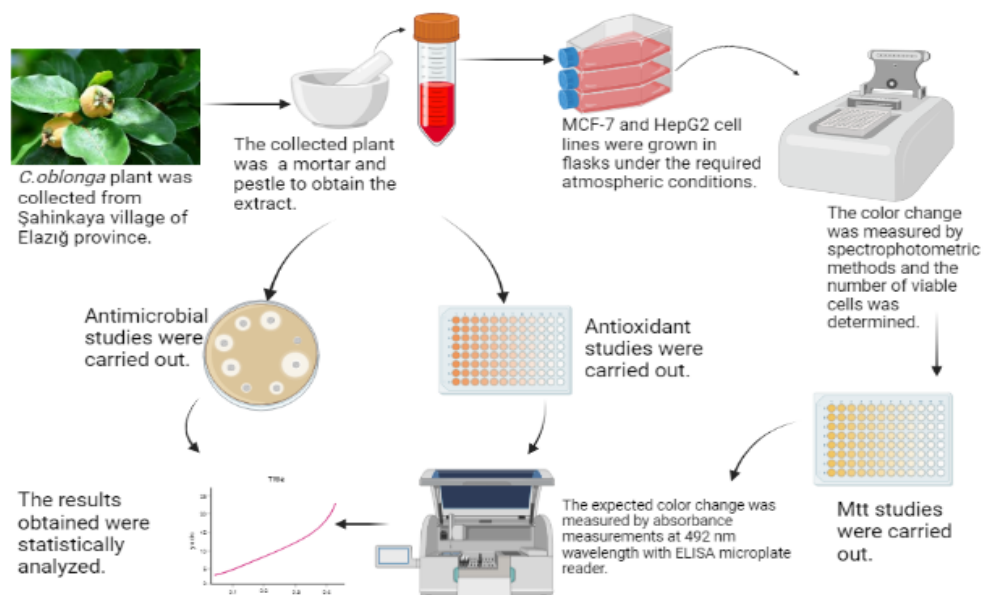


Figure 1. The overall design of the experiment showed the methods applied on *C. oblonga* leaves. Two different solvents; hexane and methanol were used to evaluate cytotoxic, antioxidant and antimicrobial activity.

2. Material and Method

2.1. Material

2.1.1. Herbal material

C. oblonga leaf was collected from Şahinkaya Village of Elazığ province in November 2022. The collected plant was kept in the laboratory of Assoc. Prof. Dr. Semih DALKILIÇ, a faculty member of the Molecular Biology and Genetics Program of the Biology Department of Firat University.

2.1.2. Microorganisms and cell lines used in the experiment

Staphylococcus aureus (*S. aureus*) ATCC 25923, *Klebsiella pneumonia* (*K. pneumonia*) ATCC 700603, *Escherichia coli* (*E. coli*) ATCC 25322, *Bacillus megaterium* (*B. megaterium*) ATCC DSM32 and *Candida albicans* (*C. albicans*) FMC17 as fungus were obtained from Fethi Sekin City Hospital central laboratory.

HepG2 and MCF-7 cancer cell lines are available in the laboratory of Assoc. Prof. Dr. Semih DALKILIÇ, one of the faculty members of the Molecular Biology and Genetics Program of the Biology Department of Firat University, and we have worked on these two cell lines.

2.2. Method

2.2.1. Extract preparation

C. oblonga leaf was pounded in a porcelain mortar and powdered. The powdered plant was weighed on a precision balance and 1 gram was taken and two separate extraction processes were carried out as 10 mL methanol and 10 mL hexane. It was incubated in a shaking oven at 37°C for 72 hours. After incubation, the extract was dried and dissolved in 10 mL Dimethyl sulfoxide (DMSO). Using Whatman No 1 filter paper, the extracts were filtered and stored at +4°C.

In order to examine the antioxidant, antimicrobial and cytotoxic activities of *C. oblonga* leaf extract, methanol, which is available in our laboratory and is a solvent frequently used in antioxidant activity studies to reveal phenolic compounds of plant extracts, and hexane, which dissolves lipophilic compounds in the plant better than many other solvents, were preferred as solvents. In addition, the fact that these two solvents have less toxicity than many other solvents is another reason why they are preferred in terms of ensuring safety in the study and working environment.

2.2.2. Cell culture

MCF-7 cells were grown in 1640 RPMI and HepG2 cells were grown in DMEM (25 mM L-Glutamine, 1% Penicillin-Streptomycin and 10% FBS (Fetal Bovine Serum)) in 75 cm² flasks at 37°C under 5% CO₂ atmosphere conditions [20]

2.2.3. MTT assay

The MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) Assay is a technique employed to evaluate the viability, proliferation, and cytotoxicity of cells. The MTT method states that MTT is a tetrazolium salt that can pass through the cell membrane, based on its reduction by active mitochondria in living cells by taking electrons inside the cell and converting them into water-insoluble purple formazan crystals [21]. The formazan crystals were then dissolved in appropriate solvents and the color change was measured spectrophotometrically to determine the number of viable cells. Cells grown in 75 cm² flasks were washed with 5 mL of sterile Phosphate buffered saline (PBS) solution by removing the medium in the flask after 90% confluency. 1 mL of trypsin EDTA (Trypsin Ethylenediaminetetraacetic acid) was added to 75 cm² flasks and incubated at 37°C in an oven containing 5% CO₂ for 2 minutes. After the cells were detached from the surface, 5 mL of RPMI was added to inactivate trypsin EDTA. Cells removed from the flask were centrifuged at 2000 RPM (Revolutions per Minute) for 5 minutes and the supernatant was removed. The cell pellet was removed by thawing with 1000 µL RPMI and cell counting was performed using a Countess II automatic cell counter. After calculations were made, cell dilution was prepared using RPMI and 5×10³ cells were seeded in 96-well plates with 100 µL RPMI per well. In the first row, only medium was used as blank, 2.5 µg/mL Doxorubicin was used as positive control and only medium was used as negative control. Then, they were incubated in an oven at 37°C with 5% CO₂ for 24 hours. After the

incubation was completed, the medium in the wells was removed and 4 different concentrations of methanol and hexane extracts of *C. oblonga* leaf prepared in RPMI (100 µg/mL, 200 µg/mL, 400 µg/mL and 800 µg/mL) were added to the cells in 6 replicates. Then the cells were incubated in an oven containing 5% CO₂ at 37°C for 72 hours. At the end of the incubation period, 10 µl of MTT solution (5 mg/mL) was added to the wells containing the cells and incubated for 4 hours at 37°C in the dark containing 5% CO₂. After incubation, the medium was removed and formazan crystals were dissolved in 100 µl DMSO. The expected color change was then determined by measuring absorbance at 492 nm wavelength using an ELISA (Enzyme Linked ImmunoSorbent Assay) microplate reader [22].

2.2.4. Determination of antioxidant activity by 2,2-Diphenyl-1-Picrylhydrazyl (DPPH) Radical Scavenging Capacity

It is a method developed to assess antioxidant activity using DPPH (2,2-diphenyl-1-picrylhydrazyl) and is used to measure the free radical scavenging capacity of antioxidants against the test. It is widely used in many herbal studies and is a fast and easy method [23]. In addition, the chemicals and spectrophotometric measuring devices required for DPPH method are available in our laboratory and have been preferred in this method antioxidant tests. Antioxidant activity of *C. oblonga* leaf extracts in methanol and hexane at different concentrations was determined by DPPH radical scavenging capacity method. A lyophilized drug solution was prepared at a concentration of 5 mg/mL using methanol and hexane. The prepared solution was diluted three times and DPPH calibration curve was obtained. At the end of the specified time, absorbances were measured at 492 nm wavelength in spectrophotometer and % inhibition values were calculated. The results were calculated according to Equation 1 [24]:

$$\text{Antioxidant activity} = \frac{\text{ControlABS} - \text{SampleABS}}{\text{ControlABS}} \times 100$$

Equation 1. Formulation used in antioxidant activity calculation

2.2.5. Antimicrobial activity

Well agar method was used for antibacterial activity and *S. aureus*, *K. pneumoniae*, *E. coli*, *B. megaterium* and *C. albicans* microorganisms were used [25]. Bacteria were grown in Nutrient Broth (Biolife Lot: HE2602) and Müller-Hinton Agar (Merck Lot: VM779137) and fungi in Malt Extract Broth (Difco) before the experimental study. In order to test the antimicrobial effects of *C. oblonga* leaf extracts diluted at 25, 50, 75 and 100 mg/mL on bacterial strains, each bacterium was inoculated into Nutrient Broth with the help of a bacterial extract. The Mc Farland setting was adjusted to a turbidity of 0.5, thus achieving a dilution of 1:10 to 107 CFU/mL. 25 mL of Müller-Hinton Agar was added to the petri dishes. Petri dishes were allowed to solidify at room temperature for 10-20 minutes. On the solidified Müller-Hinton Agar, 100 µL of bacteria were inoculated evenly and then wells of the required diameter were made in Müller-Hinton Agar with the help of an agar borer (cork-borer). Compounds prepared from four different concentrations were added to 100 µL in each well and petri dishes were incubated at 37°C for 24 hours after inoculation. Clindamycin 2 mcg (Bioanalyse Lot: 171127A) was used as a positive control and DMSO as a negative control. Inhibition zones were measured with the help of a ruler [26].

2.2.6. Statistical analysis

All results were performed with SPSS 22 for Windows and one-way ANOVA was used and $p < 0.05$ was considered significant.

3. Results

3.1. Cytotoxic activity

As a result of the experiments, it was observed that the best result was obtained at a concentration of 250 $\mu\text{g/mL}$ of *C. oblonga* leaf hexane extract used on HepG2 cancer cells, followed by the second-best result of 125 $\mu\text{g/mL}$ concentration of the same extract. It was noticed that methanol 1000, 500 and 250 $\mu\text{g/mL}$ concentrations did not show toxicity against cancer cells and in addition, as seen in Figure 2, methanol extract had less toxicity on cancer cells than hexane extract. Furthermore, as shown in Figure 3, 500 $\mu\text{g/mL}$ concentration of *C. oblonga* leaf hexane extract had the best effect on MCF-7 cancer line, while methanol extract had any effect on MCF-7 cell line at all concentrations.

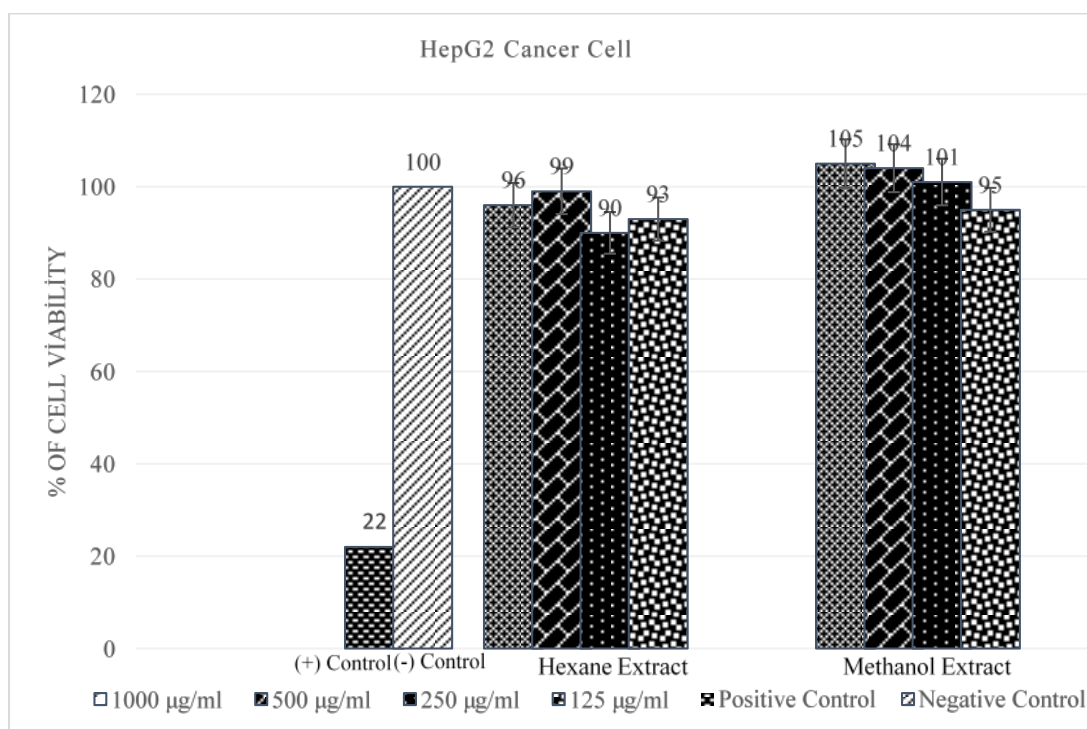


Figure 2. Cytotoxic activity of hexane and methanol extracts of *C. oblonga* leaf on HepG2 cancer cell lines. *Positive control: Doxorubicin 2.5 $\mu\text{g/mL}$, Negative control: untreated cells.

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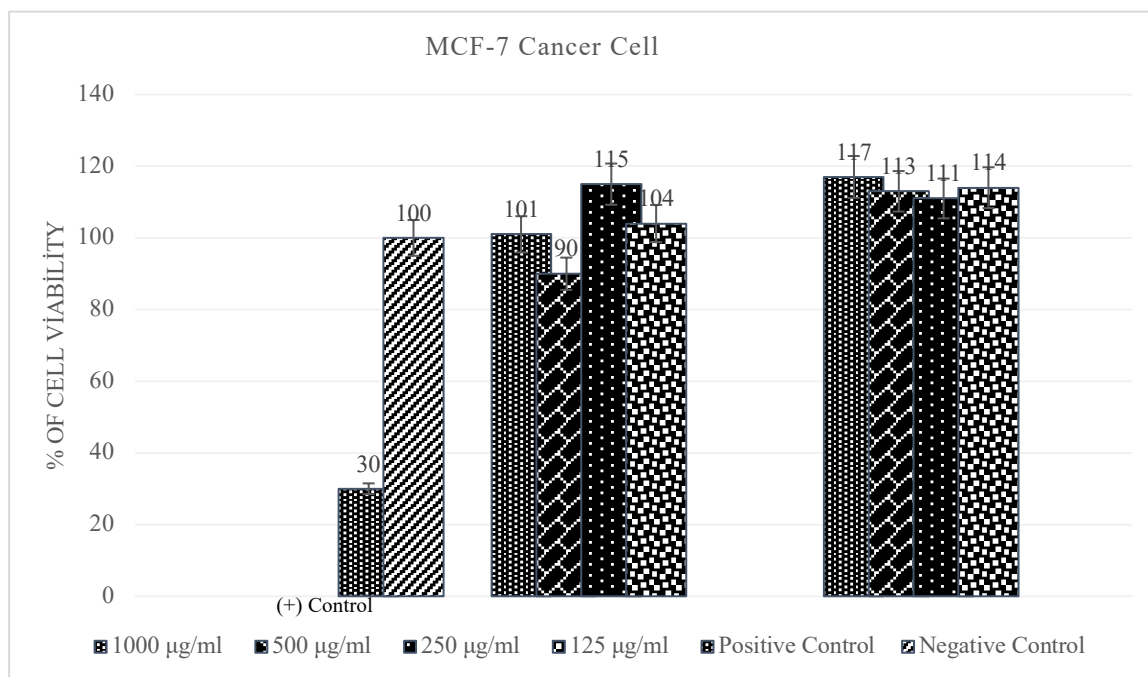


Figure 3. Cytotoxic activity of hexane and methanol extracts of *C. oblonga* leaf on MCF-7 cancer cell lines.
*Positive control: Doxorubicin 2.5 µg/mL, Negative control: untreated cells.

Cytotoxic activity results of *C. oblonga* leaf extracts were obtained by calculating the maximum inhibitory concentration (IC₅₀) value for four different concentrations (1000, 500, 250, 125 µg/mL). IC₅₀ values were calculated for both cell lines and two extracts and the results are presented in Table 1. These results differ depending on the cell line and extract type used.

Table 1. The IC₅₀ value of cytotoxic activity on cells.

MCF-7 Cell Line		
Agent	<i>C. oblonga</i> leaf hexane extract	<i>C. oblonga</i> leaf methanol extract
IC ₅₀	353.305 µg/mL	781.56 µg/mL
HepG2 Cell Line		
Agent	<i>C. oblonga</i> leaf hexane extract	<i>C. oblonga</i> leaf methanol extract
IC ₅₀	354.2317 µg/mL	139.3403 µg/mL

3.2. Antioxidant activity

Antioxidant activities of plant extracts were evaluated by DPPH method. DPPH radical inhibition percentage of *C. oblonga* leaf methanol and hexane extracts at 1000 mg/mL concentration was calculated. The results are shown in Table 2. According to the results, *C. oblonga* leaf methanol extract had 7% radical scavenging capacity and exhibited better antioxidant activity than hexane extract at a concentration of 1000 mg/mL. However, it was determined that the 1000 mg/mL concentration of *C. oblonga* leaf hexane extract did not have any scavenging capacity.

Table 2. Antioxidant activity of *C. oblonga* leaf methanol and hexane extract.

	Methanol	Hexane
Positive Control	1	1
1000 µg/ml	7	-64
Negative Control	0	0

*Positive control: 100 µL ascorbic acid and 100 µL DPPH, negative control: 200 µL DPPH.

3.3. Antimicrobial activity

The antimicrobial activity of methanol and hexane extracts of *C. oblonga* leaf plant was tested on five different microorganisms including *E. coli*, *B. megaterium*, *S. aureus*, *K. pneumonia*, *C. albicans*. In terms of antimicrobial activity, the results were compared with the standard antibiotic clindamycin. Considering these results, as seen in Table 3 and Figure 4, 50, 75 and 100 mg/mL concentrations of the hexane extract of the plant showed the highest value with an inhibition zone of 17 mm on *E. coli*, while 25 mg/mL concentration showed the lowest zone diameter effect. The best zone diameter of the extract on *S. aureus* with 16 mm was determined at a concentration of 100 mg/mL and zone diameters of 15 mm at a concentration of 75 mg/mL, 14 mm at a concentration of 50 mg/ml and 13 mm at the lowest concentration of 25 mg/mL were measured respectively. While 25 mg/mL and 50 mg/mL concentrations of the extract showed no any effect on *B. megaterium*, the 100 mg/mL concentration showed the best inhibition zone with 14 mm effect, and the 75 mg/mL concentration showed the lowest inhibition zone with 12 mm. The concentration of 100 mg/mL with 13 mm showed the best effect on *K. pneumoniae* bacteria, while the concentration of 75 mg/mL with 11 mm showed the lowest zone diameter effect. In addition, it was noticed that 25 and 50 mg/mL concentrations showed the same effect against the bacterium with 12 mm. Concentrations of 100 and 75 mg/mL were found to have the best effect on *C. albicans* with inhibition zone diameters equal to 16 mm. The lowest zone diameter of 13 mm was measured at a concentration of 25 mg/mL, followed by a concentration of 50 mg/mL with a zone diameter of 14 mm. In general, as shown in Table 3, the hexane extract showed the best antibacterial effect on *E. coli* and the lowest effect on *B. megaterium*. Looking at the methanol extract, the best inhibition zone diameter of 15 mm in *E. coli* was observed at concentrations of 75 and 25 mg/mL. Following this, the lowest zone diameter of 14 mm was measured in both 50 and 100 mg/mL concentrations. The 50 and 75 mg/mL concentrations showed the best effect against *S. aureus* with 13 mm, while the 25 mg/mL concentration showed the lowest effect with 10 mm. Following this, inhibition zone diameter of 11 mm was measured at 100 mg/mL concentration. The methanol extract, which showed the lowest effect on *B. megaterium* with 9 mm at 25 mg/mL concentration, had no effect on the bacteria at 75 mg/mL concentration, while it showed the highest effect at 50 and 100 mg/mL concentrations with 12 mm. The highest zone diameter of 11 mm is observed at concentrations of 50 and 100 mg/mL against *K. pneumoniae* bacteria. These values were followed by 25 and 75 mg/mL concentrations of 9 mm and 10 mm, respectively. At 100 mg/mL concentration, *C. albicans* showed the best zone diameter of 15 mm, at 50 and 75 mg/mL concentrations it showed the second best effect with 14 mm, but at 25 mg/mL concentration it showed the lowest effect with 12 mm. Based on these results, as shown in Table 3 and Figure 4, *C. oblonga* leaf methanol extract showed the best antibacterial effect on *E. coli* and the lowest effect on *B. megaterium*.

The standard deviations of *C. oblonga* leaves among microorganisms were calculated and as a result, *E. coli* followed by *K. pneumoniae* showed the most significant value in both extracts, while *B. megaterium* showed less significant results compared to other bacteria. Looking at the standard deviations between concentrations, it was determined that the methanol extract gave the most significant result at 50 mg/mL, followed by the hexane extract at 100 mg/mL. At 50 mg/mL, the hexane extract gave less significant results than the other results, followed by the same extract at 25 mg/mL.

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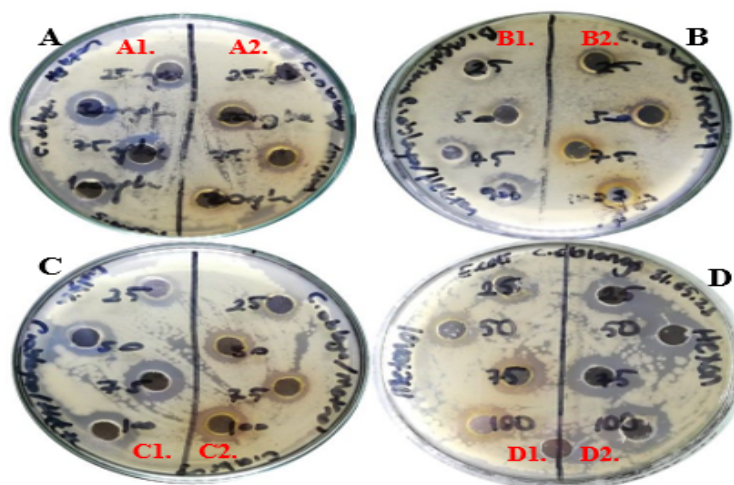


Figure 4. Zones of inhibition seen in petri dishes.

- *A: Antimicrobial effect of *C. oblonga* leaf on *S. aureus* A1: Hexane extract A2: Methanol extract
 *B: Antimicrobial effect of *C. oblonga* leaf on *B. megaterium*; B1: Hexane extract B2: Methanol extract
 *C: Antimicrobial effect of *C. oblonga* leaf on *C. albicans*; C1: Hexane extract C2: Methanol extract
 *D: Antimicrobial effect of *C. oblonga* leaf on *E. coli*; D1: Methanol extract D2: Hexane extract

Table 3. Antibacterial effect of *C. oblonga* leaf extract (zone diameters mm)

Microorganism	Concentrations (mg/mL) and zone diameters (mm)								Positive Control (Clindamycin)
	Hexane				Methanol				
	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	25 mg/mL	50 mg/mL	75 mg/mL	100 mg/mL	
<i>E. coli</i>	16 ± 0.7	17 ± 0.2	17 ± 0.2	17 ± 0.2	15 ± 0.5	14±0.5	15 ± 0.5	14 ± 0.5	24 ± 1.6
<i>S. aureus</i>	13 ± 1.5	14 ± 0.5	15 ± 0.5	16 ± 1.5	10 ± 1.7	13±1.2	13 ± 1.2	11 ± 0.7	23 ± 0.6
<i>B. megaterium</i>	*	*	12 ± 1	14 ± 1	9 ± 2	12 ± 1	12 ± 1	12 ± 1	23 ± 0.6
<i>K. pneumoniae</i>	12 ± 0	12 ± 0	11 ± 1	13 ± 1	9 ± 1.2	11±0.7	10 ± 0.2	11 ± 0.7	22 ± 0.4
<i>C. albicans</i>	13 ± 1.7	14 ± 0.7	16 ± 1.2	16 ± 1.2	12 ± 1.7	14±0.2	14 ± 0.2	15 ± 1.2	20 ± 2.4

* It did not show any antibacterial activity.

4. Discussion

C. oblonga leaf, commonly known as quince, is a good, low budget, easily accessible and health-promoting plant with antioxidant properties [12], [27], [28]. In the studies, it was determined that *C. oblonga*, which was found to have higher phenolic compound content and stronger antioxidant capacity compared to other plants used in the studies, could be valuable in nutraceuticals as an antioxidant source and would reveal new possible applications of the plant [29]. DPPH test, ABTS (2,2-azinobis(3-ethylbenzothiazollin-6-sulfonic acid) and ferric acid reducing antioxidant assays showed that *C. oblonga* had the highest antioxidant potential [30]. When the leaves of the plant and *Camellia sinensis* (*C. sinensis*) known as green tea, were compared, DPPH method was applied with methanolic extracts and as a result, EC₅₀ values were determined as 21.6 µg/mL and 12.7 µg/mL and it was statistically revealed that *C. sinensis* had a higher capacity (p<0.005). However, considering the high phenolic content of *C. oblonga* leaves compared to peel, seed or pulp, the EC₅₀ values of 600, 1700 and 2000 µg/mL for the peel were determined that the leaf would have a higher antioxidant effect (EC₅₀ 21.6 µg/mL) [31]. Quince fruit, compared to apple, is characterized by the presence of compounds such as 4-caffeoyl shikimic acid,

4-caffeoyl quinic acid, quercetin-3,7-diglucoside, kaempferol-3-O-rhamnoside and kaempferol-7-O-glucoside, while it lacks dihydrochalcone compounds. However, quince peel has the highest phenolic content, while the most abundant compound in quince pulp is 3-O-caffeoylquinic acid [29]. *C. oblonga* Mill. leaves were examined for their antioxidant activity along with their in vivo antidiabetic activity and the highest ABTS radical scavenging effect, reducing power and total antioxidant activity were observed in *C. oblonga* [32]. Parts of the plant were evaluated in three different ways: fresh frozen, oven dried and sun dried. According to the observations, fresh frozen fruit gave results close to normal nutritional values, but the oven dried plant retained antioxidant activity better than sun dried and frozen plants [33]. Methanolic extracts were prepared from various parts of *C. oblonga* plant and the bark was found to have the best antioxidant capacity [34]. *C. oblonga* leaf extract showed concentration dependent growth inhibitory activity against human colon cancer cells and no any effect against renal adenocarcinoma cells. However, seed extracts of the plant had no any effect on cell growth in colon cancer, but effective antiproliferative activity detected in kidney cancer cells [18]. When two different extracts of *C. oblonga* were extracted as lipophilic wax extract and aqueous fermented extract and their cytotoxic effects on human HepG2, A549 (human non-small cell lung cancer cell line) and HeLa cell lines were evaluated, it was determined that the aqueous fermented extract played a more effective role than lipophilic extracts, but this would vary depending on the exposure time [35]. In colorectal cancer, polyphenol-rich extracts of *C. oblonga* bark inhibited colon adenocarcinoma LS174 cells in a quantity dependent manner [36]. MTS (5-(3-carboxymethoxyphenyl)-2-(4,5-dimethyl-thiazole)-3-(4-sulfophenyl) tetrazolium, inner salt assay) testing of *C. oblonga* petals, leaves and fruit pellet on BT-20 (human breast cancer cell line), HepG2 and Caco-2 (human colorectal cancer cell line) human cancer lines showed that these three *C. oblonga* extracts strongly reduced cell growth and had low toxicity on HepG2 and Caco-2 cell lines [37]. When RD (Rabdomiyosarkom) and L2OB cancer cells were exposed to the methanol extract of *C. oblonga* seed, it was reported that it had a cytotoxic effect at the first 1000 and 500 µg/mL, but this cytotoxic activity changed depending on the concentration in the following time [38]. The antimicrobial effect of ethanol, acetone and water extracts of *C. oblonga* plant seeds on *K. pneumonia*, *E. coli*, *E. aerogenes* (*Enterobacter aerogenes*) bacteria were examined and it was determined that the seed had the most effective antimicrobial effect in the ethanolic extract and *E. coli* showed the highest sensitivity, while the aqueous extract was only effective on *E. aerogenes* bacteria [39]. With the application of *C. oblonga* seed extract to different bacterial strains, quince seed extract was found to have antimicrobial effect against gram positive *S. aureus* and gram negative *E. coli* and *P. aeruginosa* (*Pseudomonas aeruginosa*) bacteria and the MIC (Minimal inhibitory concentration) value against these bacteria was 500 µg/mL [40]. Regarding the antimicrobial effect of the plant, gram negative bacteria were more sensitive to the extracts, while the extracts obtained from *C. oblonga* flour showed growth inhibitory effect on *Aspergillus niger* (*A. niger*) [41]. To promote the full reuse of this plant, biologically active extracts and fiber concentrates of the plant were extracted and found to exhibit in vitro antioxidant activity as well as antimicrobial activity against food borne fungi and bacteria, suggesting that various extracts of the plant could be pioneers in the use of natural preservatives in foods [42]. Dye was obtained from the extract of *C. oblonga* plant in order to work on recovery to nature and dyeing process was carried out with various chemicals as a coloring agent on wool. When the properties of the extract used as a dye were tested, it has been shown to have strong antimicrobial activity on gram positive and gram negative bacteria [43]. In the study conducted with silver nanoparticles from *C. oblonga* leaves, the antimicrobial activity of these nanoparticles was examined and tested on gram negative *E. coli*, gram positive *S. aureus* and *C. albicans* and MIC values were determined as 0.0552, 0.1535 and 0.0383 mg L⁻¹ [44].

The analyzed studies showed that the leaves and various parts of *C. oblonga* showed high antioxidant activity compared to some other plants. It was observed that different solvents from the solvents used in cytotoxic activity studies may show good activity, the same solvents such as methanol used in our study showed similar effects, followed by antimicrobial activity studies also showed similar effects especially on *E. coli*, supporting the results of our study.

5. Conclusions

While hexane extract of *C. oblonga* showed better anticancer effect than methanol extract, when antioxidant activity was considered, methanol extract showed better antioxidant activity than hexane. In terms of antimicrobial activity, the best zone diameter was observed in *E. coli* bacteria and the lowest zone diameter was observed in *B. megaterium* at both concentrations. As a result, it is concluded that *C. oblonga* leaf exhibits significant anticancer, antimicrobial and antioxidant activities in an in vitro model and therefore, it may be one of the important therapeutic herbal agents.

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