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Determination of cytotoxic, apoptotic, necrotic, antimicrobial and antioxidant activities of *Aloe vera* and *Abies cilicia* subsp. *cilicica*

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ABSTRACT

Cancer is one of the most common pathologies in the world, leading to a reduced standard of living and even death for centuries. Despite promising developments in treatment methods in recent years, the expected level of treatment and success hasn't yet been achieved due to the side effects and cost of treatment methods and the fact that some drugs are still in the trial phase. This situation has encouraged the scientific community to search for natural agents with lower costs and limited side effects. *Abies cilicica*, also known as fir, and *Aloe vera* has been used in both food and traditional medicine from the past to the present. In the literature review, it was found that both *A. vera* and *A. cilicica* have many beneficial effects, especially anti-inflammatory, antifungal, and wound-healing properties. This study aimed to investigate the antimicrobial, antioxidant, cytotoxic, and apoptotic/necrotic effects of extracts of *A. vera* from Asphodelaceae and *A. cilicica* (Ant. Et Kotschy.) subsp. *cilicica* Carr. from Pinaceae. The best antimicrobial activity was observed against *Escerichia coli* with a zone diameter of 20.00 ± 3.59 mm and *Klebsiella pneumoniae* with a zone diameter of 21 ± 5.35 mm. 2KA showed the best effect on antioxidant activity. 2MA + 2KSA showed significant cytotoxic activity on MDA-MB-231 cancer cells. IC_{50} values of 1EA + 1KA extract (whole *A. cilicica* and *A. vera* dissolved in ethanol) against MDA-MB-231 cell line (IC_{50} 458.29 ± 19.01 µg/ml) and MCF-7 cell line (IC_{50} 596.03 ± 5.56 µg/ml) were determined. According to the data obtained from the study, *A. vera* and *A. cilicica* were found to have antimicrobial, antioxidant, and cytotoxic effects both alone and synergistically. It is predicted that they can be used especially in cancer treatment.

1. Introduction

Aloe vera (*A. barbadensis*) is a monocotyledonous closed-seeded plant belonging to the Asparagales order of the Asphodelaceae family. It is a well-known herbal medicine with a long history of use for its healing and therapeutic properties. Over 75 active constituents have been identified as having medicinal value and are believed to be used in combination to alleviate various diseases (Guo & Mei, 2016). *A. vera* has multiple benefits beyond just its curative properties. It has a laxative effect, is anti-microbial, stimulates the immune system, helps with wound healing, has anti-inflammatory properties, has anti-tumor effects, and can even help with diabetes. Additionally, it improves the bioavailability of vitamins and increases the absorption of poorly absorbable drugs (Nejatzadeh-Barandozi, 2013). Studies have shown that the use of low concentrations of anti-cancer agents in combination with *A. vera* components can have an even greater inhibitory effect on cancer cell growth (Fenig et al., 2004; Manirakiza et al., 2021; Wasserman et al., 2002). *A. vera* is commonly used as a nutritional supplement in various foods and as an ingredient in cosmetics. It has been claimed to have anti- inflammatory,

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antioxidant, anti-cancer, anti-aging, cardiovascular, wound healing, and immune-boosting effects (Guo & Mei, 2016; Hamman, 2008; Nejatzadeh-Barandozi, 2013). The polysaccharides found in the gel of *A. vera* are thought to have immune-boosting effects and improve phagocytosis (Guo & Mei, 2016). Lectin-like proteins are believed to contribute to its anti-inflammatory properties. In vitro studies have demonstrated that *A. vera* has an antiproliferative effect on human liver cancer cells. While *A. vera* has many beneficial effects, some studies have also reported that the plant gel may have a cytotoxic effect on human tumor cells and normal cells in vitro (Kuo et al., 2002). The green stems of *A. vera* are believed to contain high levels of anthraquinones, which have a negative effect on cell growth. Low molecular weight substances like aloin are thought to be responsible for this cytotoxicity (Avila et al., 1997; Erdogan et al., 2020).

Abies cilicica, also known as Taurus fir, is a species of fir tree native to the Taurus Mountains of Turkey. *A. cilicica*, is a conifer of the Pinaceae family. It is an evergreen, coniferous tree known for its narrow, conical shape and fragrant needles (Awad et al., 2014). *A. cilicica* is considered to be a valuable species for forestry and ornamental purposes, as well as being an important part of the ecosystem in its natural habitat (Yener, 2012). The genus Abies includes 10 species, which can be divided into two subspecies: *cilicica* and *isaurica*. The subsp. *cilicica* is characterized by having non-resinous buds and hairy young shoots, while the subsp. *isaurica* has resinous buds and smooth young shoots. *A. cilicica* (Ant. Et Kotschy.) subsp. *cilicica* Carr. is native to the Mediterranean region of Turkey. The female cones are the only part of the tree that produces resin, and the resin is found on the scales of the cones. In autumn, the cones begin to break down and fall to the ground, where the resin on the scales can be collected (Bozkuş, 1986; López-Tirado et al., 2023; Tumen et al., 2011). Resin from *Abies* trees has a long history of use in traditional medicine for various purposes. It has been used as an antiseptic, anti-inflammatory, antipyretic, antibacterial, antiviral, and anticancer agent. Additionally, it has been used to treat stomach diseases such as ulcers, dry lips, tuberculosis, and asthma, and as an ingredient in ointments and plasters to help heal wounds (Fujita et al., 1995; Tumen et al., 2011). Research has been conducted on the essential oils obtained from the resin of *A. cilicica* subsp. *cilicica*. Studies on the essential oils from the *A. cilicica* subsp. *cilicica* root and cones on the leaves have demonstrated antibacterial and antifungal properties in nine species of *Abies* (Kizil et al., 2002).

The current study focuses on investigating the in vitro cytotoxic, antimicrobial, and antioxidant effects of the combination of *A. vera* and *A. cilicica* subsp. *cilicica*. This study aimed to investigate the anticancer mechanism of these two different plant species on human breast cancer cells (MCF-7 and MDA-MB-231) and to determine whether the cells undergo apoptosis or necrosis. The study also aims to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) values.

2. Materials and methods

2.1. Sample retrieval and extract preparation

A. vera and A. cilicica were collected in their natural habitat (Onikişubat district of Kahramanmaraş) and identified by Assoc. Prof. Dr. Gülden Doğan affiliated with the Department of Biology, Fırat University. Subsequently, *A. vera* and *A. cilicica* were placed in the Herbarium of Fırat University (FUH-8893 and FUH-8894, respectively). The herbal extracts were processed by pounding them into powder in a porcelain mortar and then dissolving them in a mixture of methanol and ethanol (1 g of plants was taken and dissolved with 10 ml of solvent). The solution was kept in the dark at room temperature for 72-96 h before filtering with Whatmann No. 1 filter paper and a 0.45 µm millipore filter. The extraction was repeated 4-6 times using a Rotary evaporator at 40 °C. The obtained extract was then dissolved in DMSO and prepared at four different concentrations (250, 500, 750, and 1000 µg/ml) (Dalkılıç et al., 2022). The steps of the experiment and synergistically effective extracts are shown in (Table 1 and Figure 1).

Table 1. Preparation of pure and synergistically acting extracts[1]

	Methanol		Ethanol	
	A. vera	*A. cilicica*	*A. vera*	*A. cilicica*
1EA				+
2MA		•		
1KSA			•	
1K			•	
2KSA	•			
2K	•			
1 EA + 1 KSA			•	•
1 EA + 1 KA			•	•
2 MA + 2 KSA	•	•		
2 MA + 2 KA	•	•		

[1]**1EA:** Extract consisting of *A. cilicica* dissolved in ethanol, **2MA:** Extract consisting of *A. cilicica* dissolved in methanol, **1KSA:** Extract consisting of gel portion of *A. vera* dissolved in ethanol, **1KA:** Extract consisting of the whole part of *A. vera* dissolved in ethanol, **2KSA:** Extract consisting of gel portion of *A. vera* dissolved in methanol, **2KA:** Extract consisting of the whole part of *A. vera* dissolved in methanol. **1EA + 1KSA:** Extract consisting of *A. cilicica* and gel portion of *A. vera* dissolved in ethanol, **1EA + 1KA:** Extract consisting of *A. cilicica* and whole part of *A. vera* dissolved in ethanol, **2MA + 2KSA:** Extract consisting of *A. cilicica* and gel portion of *A. vera* dissolved in methanol, **2MA + 2KA:** Extract consisting of *A. cilicica* and whole part of *A. vera* dissolved in methanol.

2.2. Determination of antimicrobial activity

2.2.1. Microorganisms

Gram-negative (*Escherichia coli* ATCC25322 and *Klebsiella pneumoniae* ATCC700603) and gram-positive (*Bacillus megaterium* DSM32 and *Staphylococcus aureus* ATCC25923) bacteria and fungal strain (*Candida albicans* FMC17) were used in this study. Before experimental work, bacteria and fungus were grown in Nutrient Broth (Difco-Biolife Lot: HE2602) and Mueller-Hinton Agar (Merck Lot: VM779137-0xoid).

2.2.2. Preparation and application of microorganism cultures

Microorganisms were inoculated into Nutrient Broth with the help of an extract, shaken well, and incubated at 37 ± 0.1 °C for 24 h. The prepared Mueller Hinton agar was sterilized and 15-20 ml was taken and added to petri dishes. After the agar added to the petri dishes solidified, the bacteria were inoculated. 100 µl of microorganisms were added to the agar, spread homogeneously with a drigalski-spatula and wells were opened with a cork borer and 100 µl of each extract was placed in the wells. After all these procedures, the petri dishes were incubated at 37 ± 0.1 °C for 18/24 ± 2 h. At the end of the incubation period, inhibition zones formed on the medium were measured and evaluated in mm. Clindamycin (DA, 2µg/disc) and Gentamicin (CN-30, 30 µg/disc) antibiotic disks were used as positive control and 100% DMSO was used as negative control. The diameters of the inhibition zones formed at the end of the incubation period were measured with the help of a ruler and recorded (Dayisoylu et al., 2009; Dığrak et al., 1999; Eryilmaz et al., 2016). The antimicrobial effect was interpreted and analyzed by looking at the zone diameters.

2.2.3. Determination of minimum inhibition concentration (MIC)

The MIC value is a measure of the lowest concentration of an antimicrobial agent that can inhibit the growth of a microorganism. The unit of the MIC is expressed as µg/ml. The dilution method is one of the most common techniques used to determine the antibacterial activity of a substance. In this experiment, the dilutions were made using 96-well microtitration plates with a smaller amount of the antibacterial agent. 100 µl of broth and 100 µl of the extract were added to pre-prepared sterile 96-well plates (Arikan, 2007). The solution was serially diluted, and 4 µl was inoculated with three different bacteria. The plates were then incubated at 37 ± 0.1 °C for 24 h.

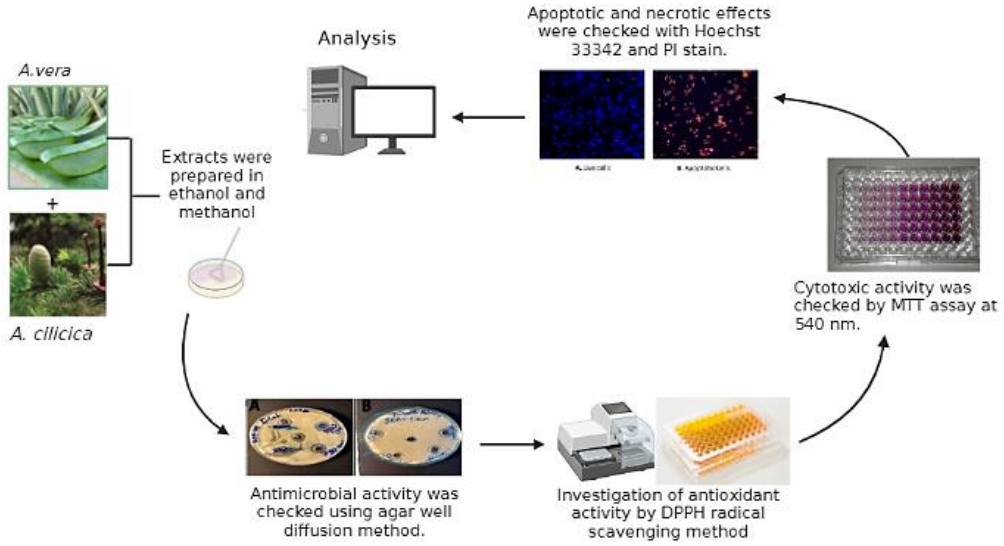

Figure 1. Experiment flow chart

The overall design of the experiment showed the methods applied to *A. vera* and *A. cilicica* plants. Two different solvents, methanol and ethanol, were used for the evaluation of antimicrobial/antioxidant/cytotoxic/apoptotic/necrotic activities.

2.2.4. Determination of minimum bactericidal concentration (MBC)

After the last inoculation and 24 h of incubation, bacterial growth on microtiter plates was determined by subculture. After 24 h, the absence of bacterial growth was used to determine the viable cell count (CFU/ml). The lowest dilution level with no growth on agar was considered MBC. A portion of the liquid (3 µl) from each well of the MIC plate was inoculated with a micropipette onto an agar plate for MBC determination. The plate was incubated at 37 ± 0.1 °C for a further 24 h (Arikan, 2007; Guzeldag et al., 2014). After subculturing, MBC was taken as the lowest concentration that showed no visible bacterial growth.

2.3. Assessment of antioxidant activity by DPPH radical scavenging method

The antioxidant activity of ethanol and methanol extract of *A. cilicica* subsp. *cilicica* and *A. vera* components were determined according to the radical scavenging capacity method of 2,2-diphenyl-1-picrylhydrazil (DPPH) at different concentrations.

All extracts were separately dissolved in methanol serially diluted using 0.005% methanolic solution of DPPH at 250, 500, 750, and 1000µg/ml concentrations. After incubating the mixtures at 37 ± 0.1 °C for 30min, the absorbance was evaluated with a spectrophotometer (540 nm). The DPPH radical scavenging rate of each sample was calculated as follows (Verbanac et al., 2016; Zeleke et al., 2020).

$$Antioxidant\ activity\ (\%) = \frac{Control_{Absorbance} - Sample_{Absorbance}}{Control_{Absorbance}} x\ 100$$

2.4. Cytotoxic activity

The cytotoxic activity was determined using human breast cancer cell lines MDA-MB-231 and MCF-7. The MDA-MB-231 cell line was cultured in DMEM with 2 mM L-Glutamine, 1% Penicillin-Streptomycin, and 10% Fetal Bovine Serum in 25/75 cm^2 flasks at 37 ± 0.1 °C and 5% CO_2. The MCF-7 cell line was cultured in RPMI with 2 mM L-Glutamine, 1% Penicillin-Streptomycin, and 10% Fetal Bovine Serum in 25/75 cm^2 flasks at 37 ± 0.1 °C and 5% CO_2. The cytotoxic activity of the substances was determined by measuring the effect of the substances on the viability of these cell lines (Dalkılıç et al., 2022). After the MDA-MB-231 cells grown in flasks became confluent, the medium was removed and washed with 5 ml of PBS solution. 1 ml of Trypsin-EDTA was added to the flasks and incubated for 2 min in an oven with 5% CO_2, 37 ± 0.1 °C. Trypsin-EDTA activation was inactivated with a 5 ml medium after the cells were separated from the surface of the flask. Cells were removed from the flask and transferred to 15 ml centrifuge tubes, centrifuged at 1300 rpm for 5 min. After centrifugation, the supernatant was removed and the cell pellet was dissolved in 1 ml of fresh medium.

Cells were counted and the number in each flask was adjusted to 5000 cells, 100 µl was inoculated into 96-well plates. Only medium was added to the first row of the plates to be used as a blank and incubated for 24 h in an oven at 37 ± 0.1 °C and 5% CO_2. After incubation, the medium was removed. 100 µl of plant extracts prepared at four different concentrations (250, 500, 750, and 1000 µg/ml) were added to the wells repeatedly and incubated for 72 h at 37 ± 0.1 °C and 5% CO_2. Subsequently, incubation, 20 µl of MTT solution was added and incubated in an oven at 37 ± 0.1 °C and 5% CO_2 for 4 h, and absorbance measurements were performed at 540 nm wavelength and doxorubicin 2.5 µg/ml were used as the positive control.

$$Live\ cell\ (\%) = \frac{A_{Sample}}{A_{Control}} x\ 100$$

2.5. Determination of apoptotic/necrotic activity using dual staining (Hoechst 33342 and Propidium iodide) method

The apoptotic and necrotic activity was determined using a dual staining method with Hoechst 33342 and Propidium Iodide (PI) dyes. The Hoechst 33342 dye penetrates the cell membrane and binds to DNA to stain the nuclei of live and dead (apoptotic/necrotic) cells. PI stain detects late apoptotic/necrotic cells because it can only enter cells with impaired membrane integrity (Karakaş, 2013). Differences in the nuclei of cells stained with Hoechst 33342 (+) and PI (-) stains were observed under fluorescence microscopy. Whether these differences lead the cells to apoptosis or necrosis can be understood by the fragmentation or shrinkage in the structure of the nucleus. If the cell nucleus shrinks, the cell goes to apoptosis, whereas if the nucleus becomes fragmented, it leads the cell to necrosis (Baran et al., 2017).

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 and discussion

3.1. Extract efficiency

As shown in Table 2, percent (%) yield calculations of the extracts were made and the yield ratios were found to be between 95-99%.

$$Efficiency\ calculation\ (\%) = (Petri\ full\ weight\ (g) - Petri\ empty\ weight)\ x\ 100$$

Table 2. Percent efficiency results of extracts[1]

Extract	Petri empty weight (g)	Petri full weight (g)	Yield result
1EA	23.08	24.07	98.81%
2MA	22.93	23.88	95.49%

[1]No loss of percentage efficiency of the extracts

Table 3. Results of antimicrobial effects (zone diameters in mm) of extracts[1]

Samples	Concentration (µg/ml)	*E. coli* (10^6 CFU/ml) (mm)	*B. megaterium* (10^6 CFU/ml) (mm)	*S. aureus* (10^6 CFU/ml) (mm)	*K. pneumonia* (10^6 CFU/ml) (mm)	*C. albicans* (10^4 CFU/ml) (mm)
1 EA	250	13 ± 1.0	12.0 ± 0.8	13.0 ± 0.5	13.0 ± 0.0	15.0 ± 1.81
	500	13 ± 1.0	13.0 ± 0.8	14.0 ± 0.5	13.0 ± 0.0	16.0 ± 1.81
	750	15 ± 1.0	13.0 ± 0.8	13.0 ± 0.5	13.0 ± 0.0	16.0 ± 1.81
	1000	13 ± 1.0	14.0 ± 0.8	16.0 ± 0.5	13.0 ± 0.0	17.0 ± 1.81
2 MA	250	18.0 ± 1.4	11.0 ± 2.51	13.0 ± 3.59	15.0 ± 3.3	14.0 ± 0.5
	500	18.0 ± 1.4	13.0 ± 2.51	15.0 ± 3.59	21.0 ± 3.3	15.0 ± 0.5
	750	19.0 ± 1.4	13.0 ± 2.51	14.0 ± 3.59	17.0 ± 3.3	15.0 ± 0.5
	1000	21.0 ± 1.4	17.0 ± 2.51	21.0 ± 3.59	22.0 ± 3.3	15.0 ± 0.5
2 KA	250	0.0 ± 4.1	8.0 ± 0.95	0.0 ± 4.19	8.0 ± 2.21	8.0 ± 4.61
	500	9.0 ± 4.1	8.0 ± 0.95	8.0 ± 4.19	12.0 ± 2.21	8.0 ± 4.61
	750	8.0 ± 4.1	10.0 ± 0.95	8.0 ± 4.19	13.0 ± 2.21	0.0 ± 4.61
	1000	8.0 ± 4.1	9.0 ± 0.95	9.0 ± 4.19	10.0 ± 2.21	0.0 ± 4.61
2 KSA	250	0.0 ± 4.61	10.0 ± 4.57	11.0 ± 1.82	11.0 ± 1.7	0.0 ± 5.74
	500	8.0 ± 4.61	0.0 ± 4.57	12.0 ± 1.82	11.0 ± 1.7	10.0 ± 5.74
	750	8.0 ± 4.61	8.0 ± 4.57	14.0 ± 1.82	12.0 ± 1.7	12.0 ± 5.74
	1000	0.0 ± 4.61	9.0 ± 4.57	15.0 ± 1.82	10.0 ± 1.7	12.0 ± 5.74
1 KA	250	11.0 ± 0.81	0.0 ± 0.0	0.0 ± 4.5	9.0 ± 0.5	11.0 ± 6.45
	500	11.0 ± 0.81	0.0 ± 0.0	0.0 ± 4.5	10.0 ± 0.5	13.0 ± 6.45
	750	11.0 ± 0.81	0.0 ± 0.0	0.0 ± 4.5	9.0 ± 0.5	14.0 ± 6.45
	1000	11.0 ± 0.81	0.0 ± 0.0	9.0 ± 4.5	9.0 ± 0.5	0.0 ± 6.45
1 KSA	250	11.0 ± 0.5	0.0 ± 4.92	10.0 ± 1.29	0.0 ± 0.0	11.0 ± 1.25
	500	11.0 ± 0.5	8.0 ± 4.92	11.0 ± 1.29	0.0 ± 0.0	11.0 ± 1.25
	750	10.0 ± 0.5	0.0 ± 4.92	12.0 ± 1.29	0.0 ± 0.0	12.0 ± 1.25
	1000	11.0 ± 0.5	9.0 ± 4.92	9.0 ± 1.29	0.0 ± 0.0	9.0 ± 1.25

[1]**1EA:** Extract consisting of *A. cilicica* dissolved in ethanol, **2MA:** Extract consisting of *A. cilicica* dissolved in methanol, **1KSA:** Extract consisting of gel portion of *A. vera* dissolved in ethanol, **1KA:** Extract consisting of the whole part of *A. vera* dissolved in ethanol, **2KSA:** Extract consisting of gel portion of *A. vera* dissolved in methanol, **2KA:** Extract consisting of the whole part of *A. vera* dissolved in methanol. One-way ANOVA was used and $p < 0.05$ was considered significant.

3.2. Antimicrobial effect

The antimicrobial properties of the extracts against four different bacteria and one fungal strain were analyzed in the study. The single and synergistic effects of the given components are interpreted individually in Table 3 and Table 4.

1EA showed activity against *C. albicans* with a zone diameter of 17 mm at a concentration of 1000 mg/ml, while it showed activity against *B. megaterium* with the lowest zone diameter of 12 mm at a concentration of 250 mg/ml. The highest inhibitory effect of the **2MA** pure component at a concentration of 1000 µg/ml was against *K. pneumoniae* (22 mm inhibition zone). At 250 µg/ml concentration, it was observed that it had the lowest effect against *B. megaterium* (11 mm inhibition zone diameter). **1KA** at a concentration of 750 µg/ml showed a high inhibitory effect on *C. albicans* with an inhibition zone of 14 mm. At the same concentration, it was found to have no effect against *B. megaterium* and *S. aureus* (0 mm inhibition zone diameter). The imported effect of **2KA** was observed against *K. pneumoniae* (13 mm inhibition zone) at a concentration of 750 µg/ml, while no effect was observed against *C. albicans* (0 mm inhibition zone diameter) at the same concentration. The most intense inhibitory effect of **1KSA** at 750 µg/ml concentration was on *S. aureus* and *C. albicans* (12 mm inhibition zone). It was determined that it did not show any effect against *B. megaterium* and *K. pneumonia* (0 mm inhibition zone diameter) at the same concentration. On the other hand, **2KSA** had the highest inhibitory effect against *S. aureus* (15 mm inhibition zone) at a concentration of 1000 µg/ml. It was observed that it did not show any effect against *E. coli* (0 mm inhibition zone diameter) at the same concentration (Table 3).

When the synergistic effects of the extracts are examined, it was observed that **1EA + 1KSA** showed the highest inhibitory effect

against *K. pneumoniae* (21 mm inhibition zone diameter) at 750 and 1000 µg/ml concentrations. These extracts showed the lowest effect (8 mm inhibition) against *C. albicans* at a concentration of 250 µg/ml. The **1EA + 1KA** component showed the highest zone diameter (15 mm inhibition zone diameter) against *E. coli* and *B. megaterium* at a concentration of 1000 µg/ml and the lowest effect (10 mm inhibition zone diameter) against *K. pneumoniae* and *C. albicans* at the same concentration) (Table 4, Figure 2). On the other hand, the **2MA + 2KA** component had the highest inhibitory effect (18 mm inhibition zone diameter) against *B. megaterium* at 750 and 1000 µg/ml concentrations, while it had the lowest inhibitory effect against *C. albicans* with 9 mm inhibition zone diameter at 1000 µg/ml concentration (Table 4, Figure 2, Figure 3). **2MA + 2KSA** component showed the highest inhibitory effect with an inhibition zone diameter of 15 mm against *B. megaterium* at a concentration of 1000 µg/ml, while it was found to have no effect against *K. pneumoniae* at the same concentration. The antimicrobial effects of the extracts and the zone diameters they formed are indicated (Table 4, Figure 3).

Table 4. Results of the antimicrobial effects of extracts by utilizing the synergistic effects[1]

Samples	Concentration (µg/ml)	*E. coli* (10^6 CFU/ml) (mm)	*B. megaterium* (10^6 CFU/ml) (mm)	*S. aureus* (10^6 CFU/ml) (mm)	*K. pneumonia* (10^6 CFU/ml) (mm)	*C. albicans* (10^4 CFU/ml) (mm)
1 EA + 1 KSA	250	13.0 ± 3.59	10.0 ± 3.3	10.0 ± 1.9	10.0 ± 5.35	8.0 ± 2.16
	500	12.0 ± 3.59	16.0 ± 3.3	12.0 ± 1.9	20.0 ± 5.35	11.0 2.16
	750	16.0 ± 3.59	17.0 ± 3.3	14.0 ± 1.9	21.0 ± 5.35	12.0 2.16
	1000	20.0 ± 3.59	12.0 ± 3.3	14.0 ± 1.9	21.0 ± 5.35	13.0 ± 2.16
2 EA + 1 KA	250	11.0 ± 1.9	14.0 ± 0.57	11.0 ± 1.73	10.0 ± 0.0	12.0 ± 1.25
	500	13.0 ± 1.9	15.0 ± 0.57	14.0 ± 1.73	10.0 ± 0.0	13.0 ± 1.25
	750	15.0 ± 1.9	14.0 ± 0.57	14.0 ± 1.73	10.0 ± 0.0	12.0 ± 1.25
	1000	15.0 ± 1.9	15.0 ± 0.57	11.0 ± 1.73	10.0 ± 0.0	10.0 ± 1.25
2 MA + 2 KA	250	10.0 ± 2.21	15.0 ± 1.73	11.0 ± 0.57	9.0 ± 1.82	12.0 ± 2.08
	500	12.0 ± 2.21	15.0 ± 1.73	11.0 ± 0.57	10.0 ± 1.82	14.0 ± 2.08
	750	14.0 ± 2.21	18.0 ± 1.73	10.0 ± 0.57	12.0 ± 1.82	11.0 ± 2.08
	1000	15.0 ± 2.21	18.0 ± 1.73	10.0 ± 0.57	13.0 ± 1.82	9.0 ± 2.08
2 MA + 2 KSA	250	11.0 ± 5.8	11.0 ± 2.0	13.0 ± 1.5	10.0 ± 5.5	10.0 ± 1.0
	500	0.0 ± 5.8	11.0 ± 2.0	10.0 ± 1.5	9.0 ± 5.5	12.0 ± 1.0
	750	10.0 ± 5.8	11.0 ± 2.0	10.0 ± 1.5	0.0 ± 5.5	12.0 ± 1.0
	1000	13.0 ± 5.8	15.0 ± 2.0	10.0 ± 1.5	0.0 ± 5.5	12.0 ± 1.0
Clindamycin (DA)		19.0 ± 0.7	18.0 ± 0.0	19.0 ± 0.0	18.0 ± 1.41	22.0 ± 4.24
Gentamycin (CN)		18.0 ± 0.7	18.0 ± 0.0	19.0 ± 0.0	20.0 ± 1.41	16.0 ± 4.24

[1]**1EA + 1KSA:** The combination of extract consisting of *A. cilicica* dissolved in ethanol and extract consisting of gel portion of *A. vera* dissolved in ethanol, **1EA + 1KA:** The combination of extract consisting of *A. cilicica* dissolved in ethanol and extract consisting of the whole part of *A. vera* dissolved in ethanol, **2MA + 2KA:** The combination of extract consisting of *A. cilicica* dissolved in methanol and extract consisting of the whole part of *A. vera* dissolved in methanol, **2MA + 2KSA:** The combination of extract consisting of *A. cilicica* dissolved in methanol and extract consisting of gel portion of *A. vera* dissolved in methanol (zone diameters in mm). One-way ANOVA was used and $p < 0.05$ was considered significant.

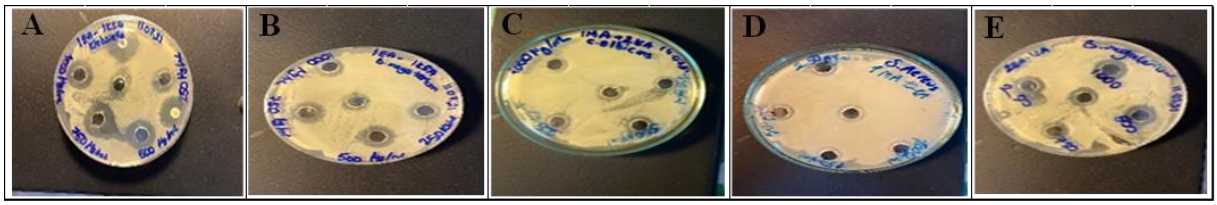

Figure 2. Inhibition zones occurred in microorganisms treated with different extracts
Antimicrobial activity of (A) **1EA + 1KSA**-*K. pneumonia*, (B) **1EA + 1KSA**-*B. megaterium*, (C) **2MA + 2KA**-*C. albicans*, (D) **2MA + 2KA**-*S. aureus*, (E) **2MA + 2KA**-*B. megaterium*

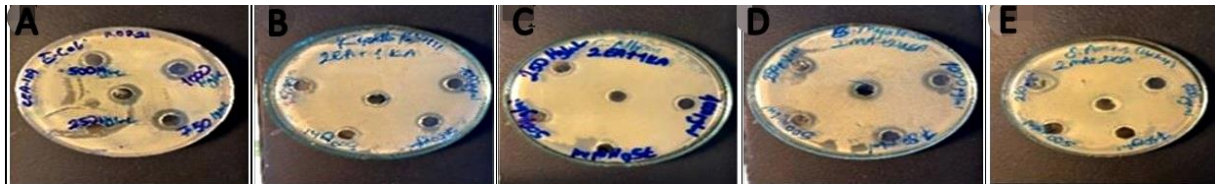

Figure 3. Inhibition zones that occur as a result of synergistic effects of the extract combinations on microorganisms
Antimicrobial effect of (A) **2EA + 1KA**–*E.coli*, B) **2EA + 1KA**-*K. pneumonia*, (C) **2EA + 1KA**-*C. albicans*, (D) **2MA + 2KSA**-*B. megaterium*, (E) **2MA + 2KSA**-*S. aureus*

In this study, the antimicrobial effects of ethanol and methanol extracts of *A. cilicica* (resin) and *A. vera* were investigated and tested for effectiveness. Since there is no study in the literature utilizing the synergistic effects of these plants, the results of the studies were compared separately. Methanol extract from *A. cilicia* leaves was active against *B. subtilis* and *S. aureus* (Dığrak et al., 1999). Studies on essential oils obtained from *A. cilicica* subsp. *cilicica* roots, leaves, and cones showed that nine *Abies* species have antibacterial and antifungal activity (Kizil et al., 2002). Methanol extract obtained from the dry leaves of *A. webbiana* was found to have broad-spectrum antimicrobial activity (Vishnoi et al., 2007). In another study, silver nanoparticles (AgNPs) synthesized from *A. vera* were reported to have strong antifungal properties against the tested *C. albicans* strains (Arsène et al., 2023). In this study, the pure component of the methanol extract of *A. cilicica* showed an inhibitory effect against *K. pneumoniae* with an inhibition zone of 22 mm when tested at a concentration of 1000 µg/ml. When the synergistic effects of the extracts were tested, it was found that the **1EA + 1KSA** mixture showed the highest inhibitory effect against *K. pneumoniae* with an inhibition zone diameter of 21 mm when tested at 750 and 1000 µg/ml concentrations. However, the same extracts showed the lowest inhibitory effect against *C. albicans* with

an inhibition zone diameter of only 8 mm when tested at a concentration of 250 µg/ml. These results suggest that the combination of **1EA + 1KSA** extracts may constitute a promising antimicrobial agent against *K. pneumoniae*, but may not be effective against *C. albicans*.

3.2.1. MIC and MBC

The MIC and MBC of the samples were determined against three different bacteria ranging from 4 to 256 µg/ml. MIC values varied depending on the test compounds (Table 5).

Table 5. Evaluation of minimal inhibitory concentration (MIC) and minimum bactericidal concentration (MBC)[1]

Samples	*E. coli*		*B. megaterium*		*K. pneumoniae*	
	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)	MIC (µg/ml)	MBC (µg/ml)
1EA	1/32	1/128	1/64	1/128	1/128	1/128
2MA	1/128	1/256	1/128	1/64	1/128	1/256
1KA	1/32	1/4	1/16	1/16	1/64	1/16
1KSA	1/32	1/8	1/16	1/16	1/64	1/32
2KA	1/32	1/4	1/128	1/128	1/64	1/32
2KSA	1/32	1/32	1/32	1/32	1/32	1/16
1EA + 1KA	1/32	1/32	1/128	1/128	1/64	1/32
1EA + 1KSA	1/32	1/16	1/128	1/32	1/32	1/32
2MA + 2KA	1/32	1/32	1/256	1/128	1/64	1/32
2MA + 2KSA	1/16	1/16	1/128	1/128	1/64	1/16

[1]Extract consisting of **1EA:** *A. cilicica* dissolved in ethanol, **2MA:** *A. cilicica* dissolved in methanol, **1KSA:** Gel portion of *A. vera* dissolved in ethanol, **1KA:** Whole part of *A. vera* dissolved in ethanol, **2KSA:** Gel portion of *A. vera* dissolved in methanol, **2KA:** Whole part of *A. vera* dissolved in methanol, **1EA + 1KSA:** *A. cilicica* dissolved in ethanol + gel portion of *A. vera* dissolved in ethanol, **1EA + 1KA:** *A. cilicica* dissolved in ethanol + whole part of *A. vera* dissolved in ethanol, **2MA + 2KA:** *A. cilicica* dissolved in methanol + whole part of *A.vera* dissolved in methanol, **2MA + 2KSA:** *A. cilicica* dissolved in methanol + gel portion of *A. vera* dissolved in methanol

Figure 4. The effects of the samples determined in the MBC test for (A) *K. pneumoniae*, (B) *E. coli* and (C) *B. megaterium*

At the MIC result, 3 µl of the 96 well plates were taken and inoculated into the designated areas on the agar plate. After inoculation, the plates were incubated for 24 h at 37 ± 0.1 °C (Figure 4).

In the MIC technique, **2MA + 2KSA** (16 µg/ml) extract showed the highest ability to inhibit *E. coli* growth while **2MA** (128 µg/ml) extract showed the lowest ability to inhibit *E. coli* growth. The highest MIC values (16 µg/ml) against *B. megaterium* were obtained from **1KA** and **1KSA** extracts, while the lowest value was **2MA + 2KA** (256 µg/ml). The highest MIC values (32 µg/ml) against *K. pneumoniae* were exhibited by the **2KSA** and **1EA + 1KSA extracts**. In the comparison of MIC results between gram-negative bacteria (*E. coli* and *K. pneumoniae*), the strongest effect was seen in *E. coli* with **2MA + 2KSA** (16 µg/ml). On the other hand, the lowest effect was observed in **2MA** extract of the same bacteria and **1EA** and **2MA** (256 µg/ml) extracts of *K. pneumoniae*. According to our results, gram-negative bacteria were more sensitive than gram-positive bacteria (Table 5). The MIC/MBC activity of ethanol and methanol extracts of *A. cilicica* and *A. vera* was tested on some microorganisms. *A. balsamea* essential oil did not affect *E. coli* and was effective against *S. aureus* with a MIC at a concentration of 56 µg/ml (Pichette et al., 2006). In another study, the antimicrobial activity of *A. vera* gel extracts against test organisms revealed the antimicrobial potency of the gel of this plant with varying zones of inhibition. The results showed that *A. vera* gel inhibited the growth of both gram-positive and gram-negative organisms and caused weak inhibition of fungi (Stanley et al., 2014). In addition, in another study, it was observed that the ethanolic extract of *A. vera* leaf gel had antibacterial activity against the test pathogens (Haque et al., 2019). The antimicrobial activities of *A. cilicica* subsp. *cilicica* were evaluated by the hydro-distillation method. According to the results of this study, it inhibited the growth of all bacteria and yeasts except *E. coli*, and *K. pneumoniae* was the most sensitive microorganism with low MIC values (Dayisoylu et al., 2009). In MBC results, *E. coli* was found to be the most sensitive pathogen with a value of 4 µg/ml in **1KA** and **2KA** extracts. **2MA** (256 µg/ml) extract against *E.coli* and *K. pneumoniae* bacteria showed the highest defense. In addition, the MBC effects of all samples against *E. coli*, *B. megaterium*, and *K. pneumoniae* ranged between 4-256 µg/ml. This result showed that the inhibition of gram-negative bacteria was stronger compared to gram-positive bacteria.

3.3. Antioxidant activity

The percentage of inhibition of DPPH radical of different concentrations of *A. vera* ve A. *cilicica* subsp. *cilicica* were shown in Figure 5. The optimization of the incubation time was performed to determine the antioxidant activity of ascorbic acid using the DPPH method.

When the antioxidant activity results are examined, **2KA** extract showed the highest rate with 67.16%.

2KSA extract has the lowest DPPH radical scavenging effect with a value of 23.21% (Figure 5).

The half-maximal inhibitory concentration (IC_{50}) measures the ability of a substance to effectively block a specific biological or metabolic function. Absorbance readings of cells treated with dimethyl-sulfoxide (DMSO) vehicle were used as control, with the percentage of cell viability in cells representing 100% survival. Using a dose-response curve and the CalcySynTM (USA) program, the cytotoxic concentrations of the extracts that achieved 50% inhibition of cell growth (IC_{50}) were determined (Al-Ghazali, 2022). The IC_{50} values for all extracts were calculated from antioxidant results **(Table 6)**.

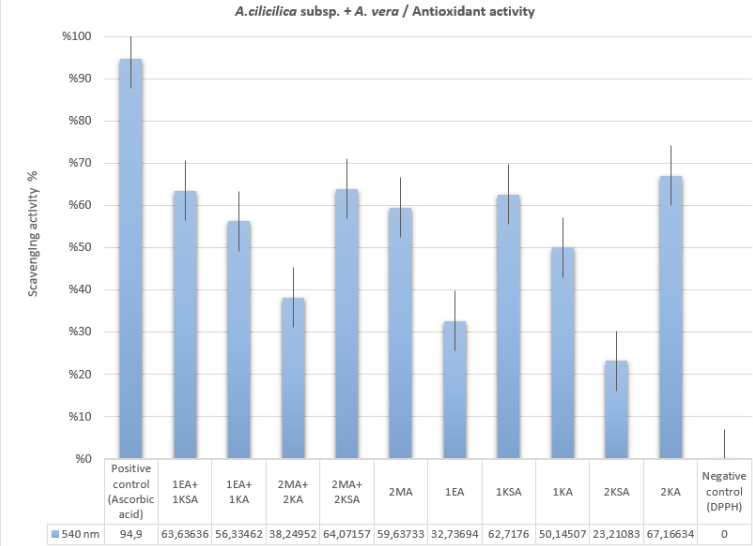

Figure 5. Antioxidant activity results of extracts by DPPH radical scavenging capacity method

Extract consisting of **1EA + 1KSA:** *A. cilicica* + gel portion of *A. vera* dissolved in ethanol, **1EA + 1KA:** *A. cilicica* + whole part of *A. vera* dissolved in ethanol, **2MA + 2KA:** *A. cilicica* + whole part of *A. vera* dissolved in methanol, **2MA + 2KSA:** *A. cilicica* + gel portion of *A. vera* dissolved in methanol, **1EA:** *A. cilicica* dissolved in ethanol, **2MA:** *A. cilicica* dissolved in methanol, **1KSA:** Gel portion of *A. vera* dissolved in ethanol, **1KA:** Whole part of *A. vera* dissolved in ethanol, **2KSA:** Gel portion of *A. vera* dissolved in methanol, **2KA:** Whole part of *A. vera* dissolved in methanol, Ascorbic acid: (+) positive control

Table 6. IC_{50} calculations of antioxidant activities of the extracts

Antioxidant activity	1EA + 1KA	1EA + 1KSA	2MA + 2KA	2MA + 2KSA	1EA	2MA	1KA	1KSA	2KA
IC_{50} (µg/mL)	984.1	473.1	307.3	275.2	1339.1	488.5	993.8	467.6	0.35

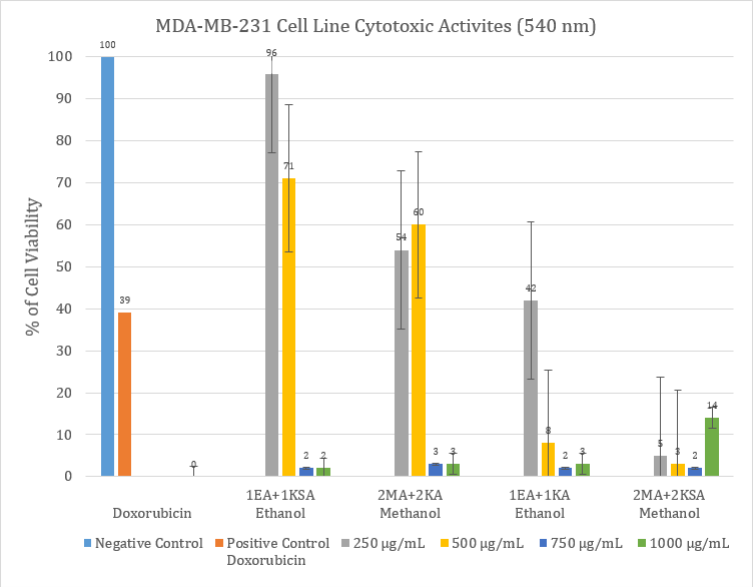

Figure 6. Cytotoxic activity of extracts' synergistic effects against MDA-MB-231 cell lines

Extract consisting of **1EA + 1KSA:** *A. cilicica* + gel portion of *A. vera* dissolved in ethanol, **1EA + 1KA:** *A. cilicica* + whole part of *A. vera* dissolved in ethanol, **2MA + 2KA:** *A. cilicica* + whole part of *A. vera* dissolved in methanol, **2MA + 2KSA:** *A. cilicica* + gel portion of *A. vera* dissolved in methanol. Each extract was tested in duplicate against cell lines in triplicate. The data show the mean of two separate experiments ($p < 0.05$).

In a study on *A. vera*, it was found that the administration of mice with *A. vera* fed a high-fat diet prevented cell damage caused by oxidative stress and dyslipidemia. It has also been reported to increase the activity of some enzymes such as superoxide dismutase and catalase, which help protect cells from damage caused by oxidative stress (Abubakar et al., 2022). According to a study,

extracts from *A. vera* leaves at a concentration of 23 µg/ml were found to inhibit the growth of the MCF-7 cancer cell line by IC_{50}, which is significantly lower than the IC_{50} value against non-cancerous cells (NIH-3T3) found at 332 µg/ml. In another study, it was reported that *A. vera* leaf extract has a high antioxidant capacity as it can scavenge free radicals, which are unstable molecules that can damage cells and lead to various diseases. The free radical scavenging activity of *A. vera* leaf extract was found to be between 67% and 89% at concentrations of 50 to 300 µg/ml. This suggests that *A. vera* leaf extract has the potential to prevent or reduce oxidative stress and related diseases caused by free radicals (Majumder et al., 2020).

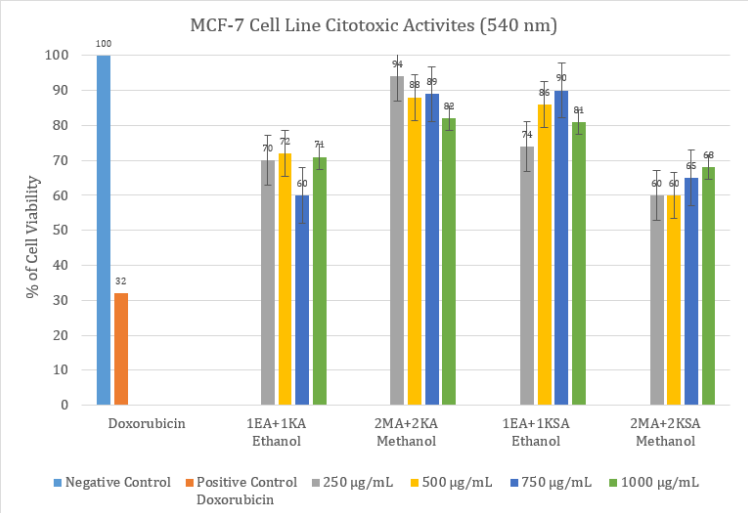

Figure 7. Cytotoxic activity of extracts against MCF-7 cell lines

Extract consisting of **1EA + 1KSA:** *A. cilicica* + gel portion of *A. vera* dissolved in ethanol **1EA + 1KA:** *A. cilicica* + whole part of *A. vera* dissolved in ethanol, **2MA + 2KA:** *A. cilicica* + whole part of *A. vera* dissolved in methanol, **2MA + 2KSA:** *A. cilicica* + gel portion of *A. vera* dissolved in methanol. Each extract was tested in duplicate against cell lines in triplicate. The data show the mean of two separate experiments ($p < 0.05$).

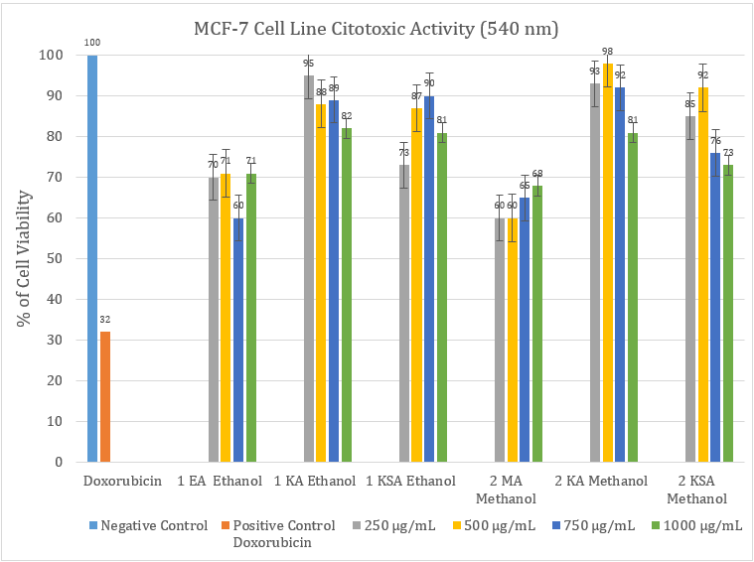

Figure 8. Cytotoxic activity of extracts against MCF-7 cell lines

Extract consisting of **1EA:** *A. cilicica* dissolved in ethanol, **2MA:** *A. cilicica* dissolved in methanol, **1KSA:** Gel portion of *A. vera* dissolved in ethanol, **1KA:** Whole part of *A. vera* dissolved in ethanol, **2KSA:** Gel portion of *A. vera* dissolved in methanol, **2KA:** Whole part of *A. vera* dissolved in methanol. Each extract was tested in duplicate against cell lines in triplicate. The data show the mean of two separate experiments ($p < 0.05$).

3.4. Cytotoxic activity

The data in Table 7, Figure 6 and Figure 7 show the percent synergistic activity of the extracts against MDA-MB-231 and MCF-7 cell lines. 1000 µg/ml and 750 µg/ml concentrations of **1EA + 1KSA**, **2MA + 2KA**, and **1EA + 1KA** extracts showed high cytotoxic activity against the MDA-MB-231 cell line, and these effects were determined between 95% and 98%. No effects have been reported at lower concentrations (Figure 6).

1EA + 1KA, **1EA + 1KSA**, and **2MA + 2KSA** extracts showed cytotoxic effects on MDA-MB 231 cell lines between 40% and 32% at all concentrations (Figure 7).

2MA and **1EA** extracts showed the best cytotoxic effect against MCF-7 cell line and was destroyed 40% of MCF-7 cells. In **1KA**, **2KA**, **1KSA**, and **2KSA** extracts, the cell killing rate varies between 28% and 37% (Figure 8).

It has been reported that the methanol extract obtained from the leaves of *A. cilicia* produced an inhibition zone diameter of 10-12 mm against *B. subtilis*, *S. aureus*, and *K. pneumoniae* bacteria (Dığrak et al., 1999). The cytotoxic activity of *A. cilicica* resin extract, which was prepared with hexane and chloroform, against human cervical cancer cells and C6 rat brain tumor cell lines was investigated. It was observed that the cytotoxic activity increased depending on the concentration (Yaglioglu et al., 2022).

Table 7. Cytotoxic effects of extracts on MDA-MB-231 and MCF-7 cell lines[1]

Extracts	Cell lines (viability, %)		
	Dose (µg/ml)	MDA-MB-231	MCF-7
1EA + 1KSA	250	96	*
	500	71	*
	750	2	*
	1000	2	*
1EA + 1KSA	250	96	*
	500	71	*
	750	2	*
	1000	2	*
2MA + 2KA	250	54	94
	500	60	88
	750	3	89
	1000	3	82
1EA + 1KA	250	42	70
	500	8	72
	750	2	60
	1000	3	71
2MA + 2KSA	250	5	60
	500	3	60
	750	2	65
	1000	14	68
1EA	250	*	70
	500	*	71
	750	*	60
	1000	*	71
1KA	250	*	95
	500	*	88
	750	*	89
	1000	*	82
1KSA	250	*	73
	500	*	87
	750	*	90
	1000	*	81
2MA	250	*	60
	500	*	60
	750	*	65
	1000	*	68
2KA	250	*	93
	500	*	98
	750	*	92
	1000	*	91
2KSA	250	*	85
	500	*	92
	750	*	76
	1000	*	73
Doxorubicin	2.5 ng/ml	39	32
Negative control	0	100	100

3.5. Apoptotic/necrotic activity detection

Hoechst and PI fluorescent dyes were used to visualize and analyze the nuclear morphology and membrane integrity of cells, respectively. Hoechst staining is used to stain both living and dead cells, while PI staining only stains damaged cell membranes. In this study, the cells in the area indicated by the yellow marker were necrotic, but the cells in the area indicated by the red arrow underwent apoptosis. It was observed that the number and density of cancer cells decreased in the area where doxorubicin was used as a positive control. There were only DMEM and cancer cells as negative control and no treatment was applied **(Figure 9)**.

A. vera contains many important components such as aloe-emodin, aloin, chrysophanol, aloesaponarin I, aloesaponarin II, acemannan, aloesin, umbelliferone, and esculetin. The effect of these components on various cancer cell lines was examined. Aloe-emodin showed IC_{50} values of 9.87 µM to acute lymphoblastic leukemia, chrysophanol demonstrated IC_{50} values of 1.27 µM to MCF-7. The IC_{50} value of aloesaponarin II against cervical cancer was 0.98 µM, the IC_{50} value of aloesaponarin II against ovarian cancer was 5 µM, and the IC_{50} value of umbelliferone against Hep G2 cancer cell line was 5 µM (Majumder et al., 2019). *A. vera barbadensis* extract C (AVBEC) has a higher potential to induce apoptosis in cancer cells compared to normal cells. It is concluded that it achieves this property by reducing ATP concentration in cancer cells and consequently increasing ROS production. It was observed that AVBEC extract prepared at a concentration of 10 mg/ml induced apoptosis in various cancer cell lines including MCF-7 cells, MDA-MB-231, NCI-H 524, and NCI-H 1975, but lower concentrations of AVBEC (2.5 mg/ml and 5 mg/ml) led the cells to apoptosis in MCF-7 and NCI-H 524 cell lines. This suggests that it

may have an apoptosis-inducing effect depending on the concentration and the type of cell lines (Tong et al., 2021). The antimicrobial activity of *A. vera* leaf extract against *Candida* (*kefyr*, *glabrata*, and *albicans*) at varying concentrations was investigated. The highest effect against *Candida* species was observed on *C. glabrata* at a concentration of 400 mg/ml (33.00 ± 3.61 mm) (Al-Ghazali, 2022).

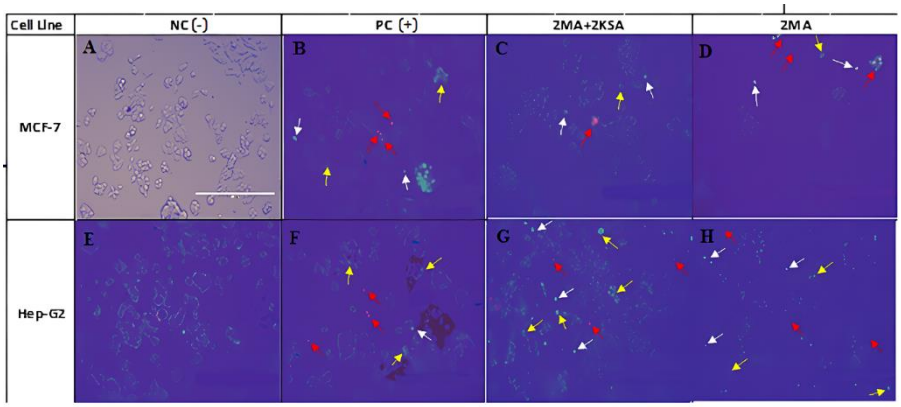

Figure 9. Apoptotic and necrotic effects of extracts used against cancer cell lines

(A) Negative control of MCF-7 cell line from control groups, (B) Doxorubicin-treated positive control (+) of the MCF-7 cell line from the control group, (C) The experimental group of MCF-7 cell lines to which **2MA + 2KSA** extract was applied, (D) Experimental group that applied **2MA** extract from the experimental groups of the MCF-7 cell line, (E) Negative control (-) of the Hep-G2 cell line from the control group, (F) Doxorubicin-treated positive control (+) of the Hep-G2 cell line from the control group, (G) Experimental group that applied **2MA + 2KSA** extract from the control groups of the Hep-G2 cell line, (H) The experimental group of Hep-G2 cell lines to which **2MA** extract was applied, Images were taken with a fluorescent inverted microscope at 10x magnification. Cells were stained with Hoechst 33258 (blue) and PI (red) to visualize cell death. White arrows indicate pycnosis, yellow arrows decomposed nuclei (necrotic), and red arrows indicate apoptotic cells.

The inflammatory effect of *Cedrus libani* and *A. cilicica* subsp. *cilicica* essential oils on the wound model were studied at a dose of 200 mg/kg and a dose-dependent activity was observed with inhibition values of 29.8% and 30.5% ($p < 0.01$), respectively (Tumen et al., 2011).

4. Conclusions

At present, both cytotoxic and apoptotic/necrotic activity investigations of plant extracts are very important. Therefore, the cytotoxic activities of *A. cilicica* and *A. vera* on MCF-7 and Hep-G2 cell lines, as well as their apoptotic/necrotic activities and antimicrobial activities, were evaluated. As a result, it was reported that the cytotoxic effects on cell lines are synergistic when the two plant extracts are used in combination. In the future, plant extracts and their constituents can be used promisingly as adjuvant drugs alone or synergistically in the treatment of cancer. However, further studies are needed before plant extracts can be studied in vitro and in vivo in animal models.

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Conflict of interest

The authors confirm that there are no known conflicts of interest.

Statement of ethics

In this study, no method requiring the permission of the "Ethics Committee" was used.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

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Supplementary File

None.

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