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Article

Investigation of Cytotoxic, Antimicrobial and Antioxidant Activities of *Echium vulgare* L. Seed

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Abstract: *Echium vulgare* L. is a plant belonging to the Boraginaceae family and is used in traditional medicine for the treatment of various diseases, especially known for its anti-inflammatory and antioxidant properties. The aim of this study was to determine the cytotoxic activity of different concentrations of *E. vulgare* L. seed extracts obtained from methanol and hexane solvents on human breast cancer (MCF7) and human liver cancer (HepG2) cell lines using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The antioxidant activity of the extract obtained from methanol and hexane at the same concentrations was determined according to the 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging capacity method. Antimicrobial activity was determined by disk diffusion method and bacteria such as *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Escherichia coli* and *Bacillus megaterium* and *Candida albicans* microorganisms as fungi were used for the study. The highest antibacterial activity was detected against *Escherichia coli* (*E. coli*) with a zone diameter of 16±4 mm. Seed extract of *E. vulgare* prepared with hexane showed the most effective cytotoxic activity on HepG2 cell line with 20% cytotoxicity rate. In antioxidant activity tests, the methanol extract of *E. vulgare* exhibited the highest activity with 4.5% reduction. In conclusion, seed extracts of *E. vulgare* appear to be a potential source for both anticancer and antimicrobial therapies.

Key words: *Echium vulgare,* Cytotoxic, Antimicrobial, Antioxidant activity, MCF7, HepG2 cell lines

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1. Introduction

Cancer is one of the most serious health, social and economic problems worldwide and in many places it is the first or second main cause of death (Peña-Corona et al., 2023). Recently, significant progress in our understanding of molecular and tumor biology has led to a remarkable transformation in cancer treatment approaches. In the past, cancer was only categorized and treated based on the organs of origin or basic histomorphological features. However, a major breakthrough occurred when researchers observed improved survival rates in cancer patients following the administration of four different combinations of platinum based chemotherapy including third generation drugs, marking a turning point in cancer treatment strategies (Zugazagoitia et al., 2016). Despite encouraging advances in cancer diagnosis and treatment, the challenges in managing the disease and the serious side effects of drugs that significantly affect patients' quality of life have prompted the scientific community to explore new therapeutic approaches (Farràs et al., 2021). Over the years, medicinal plants have been favored both as an origin of food and for traditional medicine due to their important biological conditions (Gecer & Erenler, 2023). More than 60% of plant derived drugs have anti cancer effects and cause fewer side effects in treatment (Shin et al., 2018). The perennial herb *Echium vulgare* is a member of the Boraginaceae family's Echium genus. It performs best when growing naturally in areas with plenty of sunlight, fields, overgrazing pastures, poor draining valleys, and roadside locations (Kapusterynska et al., 2020). Echium species exhibit various life cycles as annual, biennial or perennial in their natural habitats and are known for their beautiful flowering appearance (Jin et al., 2020). Studies have reported antibacterial, anti-inflammatory, antiproliferative, antidepressant, antioxidant, antiviral and cytotoxic activities (Alsanie et al., 2018). *E. vulgare* flowers are frequently visited by honeybees as they are a source of nectar and pollen. The leaves are used in cooking, as an expectorant and anticovulsant for epilepsy. Furthermore, *E. vulgare* seed oil is recognized for its high content of omega-3 or omega-6 polyunsaturated fatty acids (PUFA), including g-linolenic acid (GLA) and stearidonic acid (SDA) (Kapusterynska et al., 2020). *E. vulgare* is rich in oligophenols and

pyrrolizidine alkaloidsIts chemical makeup has led to a lengthy history of application in both conventional and veterinary medicine. This is a factor that has made it valuable in many fields for many years (Alsanie et al., 2018). In traditional folk medicine, *E. vulgare* is valued for its ability to treat cracked hands and promote wound healing. In addition to these, this plant knows as a significant source of wild honey (Klemow et al., 2002). *E. vulgare* is grown mainly in Turkey. Both Turkish and German folk traditions have used the roots of *E. vulgare* for a long time to treat hand cracks, hasten the healing of wounds, and soothe sprains and bruises. Additionally, the flowers and leaves of *E. vulgare* are valued for their cough relieving and diuretic properties, and the plant is recognized as a significant source of wild honey (Kuruüzüm-Uz et al., 2004). This study aimed to ascertain the antibacterial activity of *E. vulgare* seed against microorganisms as well as its cytotoxic and antioxidant properties on the cell lines of liver cancer (HepG2) and breast cancer (MCF7).

2. Materials and Methods

2.1. Materials

2.1.1. Obtaining of material

Echium vulgare seeds were obtained commercially from Farmer Life, Balıkesir, Turkey. The material was identified by Assoc. Prof. Dr. Gülden Doğan.

2.1.2. Microorganisms and cell lines used in the experiment

Staphylococcus aureus ATCC 25923, *Klebsiella pneumoniae* ATCC 700603, *Esherichia coli* ATCC 25322, *Bacillus megaterium* ATCC DSM32 and *Candida albicans* FMC17 as fungus were obtained from Fethi Sekin City Hospital central laboratory. Human liver cancer HepG2 (Passage:8) and breast cancer MCF7 (Passage:7) cell lines are available in the laboratory of Assoc. Prof. Dr. Semih Dalkiliç, a faculty member of the Molecular Biology and Genetics Program of the Department of Biology at Firat University. These cell lines were used in this study.

2.2. Methods

2.2.1. Extract preparation

E. vulgare seeds were pulverized by crushing with a pestle in a porcelain mortar. Two separate extracts were prepared with 10 mL of methanol and hexane by taking 1 gram of the powdered seeds on a precision balance. In a shaking oven, the extracts were incubated for 72 hours at 37 ˚C. After drying, the extracts were dissolved in 10 mL Dimethyl sulfoxide (DMSO) and filtered with filter paper (Whatman No 1). They were kept at +4 °C. To assess the antioxidant, cytotoxic and antibacterial properties *E. vulgare* seed extract, hexane, which is widely used in the determination of phenolic synthetics and methanol, which effectively solubilizes normal lipophilic treatments, were preferred. The fact that these two solvents are less toxic compared to other solvents makes them more attractive in terms of the safety of the working process and the laboratory environment.

2.2.2. Antimicrobial activity

Agar well method was used for antibacterial activity evaluations and *S. aureus, K. pneumoniae, E. coli, B. megaterium* and *C. albicans* microorganisms were used in this method (Dalkiliç et al., 2023a). Microorganisms were added to Nutrient Broth. The Mc Farland adjustment was set to 0.5 turbidity and a dilution of 107 CFU/ml was obtained. Mixed thoroughly and then incubated at 37±0.1 °C for 24 hours. Meanwhile, Mueller Hinton agar was prepared and sterilized, after which 15-20 mL of this agar was dispensed into individual in petri dishes. After the agar in the petri dishes solidified, the bacteria were inoculated onto the surface. Specifically, 100 µL of the microorganism culture was evenly spread across the agar using a drigalski. Wells were then created in the agar using a cork borer and 100 µL of each extract of interest was dropped into these wells. Following these procedures are, the petri dishes were then placed into an incubator set at a temperature of 37±0.1 °C for a period of 18/24±2 hours. At the end of the incubation period, any zones of inhibition that had formed on the agar surface were measured and assessed in millimeters (mm). A positive control utilized Clindamycin antibiotic disks (2 mcg), while a negative control employed 100% DMSO. The sizes of the inhibition zones formed measured in at the conclusion of the incubation time using a ruler and recorded (Dalkılıç et al., 2023b).The antimicrobial impact was determined and assessed by examining the sizes of the inhibition zones.

2.2.3. Cell culture

HepG2 cells were grown in DMEM containing 10% fetal bovine serum (FBS), 1% Penicillin-Streptomycin and 25 mM L-glutamine and MCF7 cells were grown in 1640 RPMI. Cells were cultured in 75 cm^2flasks at 37 °C with 5% CO_2. (Silva de Carvalho et al., 2022).

2.2.4. Determination of cytotoxic activity

Cytotoxic activity was assayed using MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) tetrazolium test (Grela et al., 2018). The MTT method operates on the principle that functional mitochondria within living cells reduce MTT, a tetrazolium salt capable of permeating the cell membrane. This reduction process involves the transfer of electrons within the cell, leading to the formation of water insoluble purple formazan crystals (Dalkilic et al., 2021; Dalkılıç et al., 2023b). When cells in 75 cm^2 flasks reached 90% confluence, the media was withdrawn, and the cells were cleaned with 5 mL of sterile Phosphate Buffered Saline (PBS). Subsequently, 1 mL of Trypsin EDTA was introduced into these flasks, and they were incubated at 37 °C under 5% CO_2 for a duration of 2 minutes. Once the cells were detached from the surface, 5 mL of RPMI was added to halt the trypsin activity. The cells were then discarded the flask then centrifuged at 2000 RPM (Revolutions Per Minute) for 5 minutes. Following the removal of the supernatant, 1000 µL of RPMI was used to suspend the pellet. The cell count was then analyzed using a Countess II automated cell counter. Subsequently, cell counting was carried out using an automated Countess II cell counter. The cells were then seated into 96 well plates at a density of 5×10^3 cells per well and incubated overnight. The next day, after removing the medium, the first row and the negative control were treated with medium only as blank, the positive control was treated with 2.5 µg/mL Doxorubicin and different concentrations of *E. vulgare* seed (100-200-400-800 µg/mL). The wells were incubated for a period of 72 hours at 37 °C in an oven under 5% CO_2 conditions. After the first 72 hours of incubation, 10 µL of MTT solution (5 mg/mL) was added to each well and the cells were incubated for 4 hours at 37°C in the absence of light. After

this incubation period, the plate was shaken for MTT solubilization and the absorbance at 517 nm wavelength was measured by ELISA (Enzyme-Linked ImmunoSorbent Assay) using a microplate reader. Cytotoxicity was calculated in this way:

$$\text{Cytotoxic activity } (\%) = \frac{\text{Sample absorbance}}{\text{Control absorbance}} \text{ x } 100$$

2.2.5. Determination of antioxidant activity

The antioxidant activity of methanol and hexane extracts of *E. vulgare* seeds was evaluated by 2,2-diphenyl-1-picrylhydrazyl (DPPH) radical scavenging effect. To evaluate the antioxidant activity of methanol and hexane extracts of *E. vulgare* seeds, the radical scavenging effect of DPPH was evaluated. The antioxidant activity of the plant extract was determined using the stable DPPH radical according to the method of Hatano et al. (1998). Briefly, 0.25 mM DPPH radical solution (0.5 ml) was added to the sample solution in ethanol (1 ml) at different concentrations of the extracts (12.5-100 µg/ml). The mixture was carefully shaken and kept in the dark for 30 minutes and the absorbance was measured at 517 nm. DPPH radical scavenging capacity was calculated using the following equation. Following a 30 min incubation at 37±0.1 °C, the absorbance of the mixtures was recorded using a spectrophotometer configured at 517 nm. The following formula was used to determine the rate at which DPPH radicals were scavenged for each mixture (Hatano et al., 1988).

$$\text{Antioxidant activity } (\%) = \frac{\text{Control absorbance} - \text{Sample absorbance}}{\text{Control absorbance}} \text{ x } 100$$

2.2.6. Statistical analysis

Data collected from the test extracts were statistically analyzed using one-way analysis of variance (ANOVA) and then evaluated at $p<0.05$ significance level using the least significant difference (LSD) test. SPSS statistical program for Windows (Version 22, SPSS Inc., Chicago, IL, USA) was used to perform these statistical procedures.

3. Results

3.1. Extract efficiency

E. vulgare seed was initially dried to 5 g in case of a replicate study. One g of the dried plant was taken and prepared separately in 10 ml of solvent (methanol and hexane). After 72 hours in a shaking oven, it was filtered with Whatman No.1 filter paper. The methanol and hexane solvents were removed in a rotary evaporator at 40-50 °C for 4 hours and dissolved in DMSO to prepare four different concentrations (100, 200, 400, 800 µg/ml) and stored at +4 °C. Percent yield calculations were made during the extraction of the extract (Kaptaner İğci & Aytaç, 2020) and there was no yield loss as shown in Table 1.

$$\text{Percent efficiency } \left(\frac{\text{w}}{\text{w}}\right) = \frac{(\text{weight of dried extract, g})}{(\text{weight of dry seed material before extraction, g})} \text{ x } 100$$

Table 1. Results of extracts regarding percent efficiency.

Extracts	Dry seed material before extraction (g)	Extract after extraction (g)	Yield efficiency percentage (%)
Hexane	1	0,0332	3,32
Methanol	1	0,0797	7,97

* No loss of percentage efficiency of the extracts.

3.2. Antimicrobial effect

Different concentrations of *E. vulgare* seed extracts prepared with methanol and hexane were evaluated for their antimicrobial activity against five microorganisms and the findings are shown in Table 2. Finally results were compared with clindamycin, standard antibiotic.

Table 2. Antimicrobial effect of hexane and methanol extracts of *E. vulgare* seed extract on *K. pneumaniae, C. albicans, S. aureus, C. albicans* and *E. coli* (zone diameters mm).

	Concentrations (mg/mL) zone diameters (mm)								
	Hexane (mg/mL)				Methanol (mg/mL)				Clindamisin (2 mcg)
Microorganism	25	50	75	100	25	50	75	100	
E. coli	9±2	11	13±2	11±2	8±4	11±1	13±1	16±4	24±1.6
S. aureus	14±1.7	14±1.7	13	12±1.2	11±1.5	12±0.5	13±0.5	14±1.5	23±0.6
B. megaterium	12	11±1	13±1	12	9±0.75	12±2.2	10±0.2	8±1.7	22±0.4
K. pneumoniae	12±0.7	13±0.2	14±1.2	12±0.7	9±2	12±1	12±1	11	23±0.6
C. albicans	9±1.2	9±1.2	11±0.7	12±1.7	*	12±4	9±1	11±3	20±2.4

*: Does not form an inhibition zone.

It was observed that the methanol extract of *E. vulgare* seed created the highest diameter of zone of 14 mm against *S. aureus* at a concentration of 100 mg/mL, while it created the lowest zone diameter (11 mm) at a concentration of 25 mg/mL. Also it was observed that the zone diameters of the methanol extract against *E. coli* at concentrations of 25 mg/mL, 50 mg/mL and 75 mg/mL were 8, 11 and 13 mm, respectively, and the best zone diameter was found to be 16 mm at the concentration of 100 mg/mL. Although it showed the lowest zone diameter (8 mm) at the highest concentration (100 mg/mL) against *B. megaterium* bacteria, the zone diameter was found to be 10 mm at a concentration of 75 mg/mL.. It was observed that the extract formed a zone diameter of 12 mm at 50 mg/mL and 75 mg/mL concentrations, 9 mm at 25 mg/mL concentration and 11 mm at 100 mg/mL concentration on *K. pneumaniae*.. While it did not show any effect on *C. albicans* at 25 mg/mL and 50 mg/mL concentrations, it showed maximum effect by forming 11 mm zone diameter at 100 mg/mL concentration. In general, Table 2 shows that the hexane extract showed maximum antibacterial effect on *S. aureus* and *K. pneumaniae*. In addition, the highest zone diameter on *E. coli* bacteria was found at a concentration of 75 mg/mL with 13 mm, while the lowest zone diameter was found at a concentration of 25 mg/mL with 9 mm. Following this, a zone diameter of 11 mm was measured at 50 and 100 mg/mL concentration. The hexane extract showed minimum activity on *B. megaterium* at 50 mg/mL concentration (11 mm) and maximum antimicrobial activity at 100 mg/mL concentration (14 mm). Hexane extract showed the lowest activity against the zone diameter of *C. albicans* with 9 mm at 25 and 50 mg/mL concentrations, 11 mm at 75 mg/mL and 10 mm at 100 mg/mL.

3.3. Cytotoxic activity

As a result of the experiments, it was observed that the methanol extract of *E. vulgare* seed showed a 14% cytotoxicity effect on HepG2 cell line at 800 µg/mL concentration compared to other concentrations. Compared to 800, 400 and 100 µg/mL concentrations of hexane extract, 200 µg/mL concentration showed 20% toxicity against cancer cells. Furthermore, as shown in Figure 2, all concentrations of *E. vulgare* seed extract prepared with hexane did not show cytotoxic activity against MCF7 cancer cell line. While no toxicity was observed at concentrations of 200 and 100 µg/mL of the methanol extract, 9% and 7% cytotoxic effect was observed at concentrations of 800 and 400 µg/mL, respectively. Considering these results, in general, 200 µg/mL concentration of hexane extract of *E. vulgare* seed had the best effect on HepG2 cell line as shown in Figure 1, while hexane extract had no effect on MCF7 cell line at all concentrations. As a result, it is observed that the tested extracts do not have a significant cytotoxic effect at the concentrations tested. Statistical analysis was performed by one-way ANOVA test and cytotoxic activity analysis using SPSS version 22. Cytotoxic activity was not considered significant because the data obtained were greater than $p<0.005$.

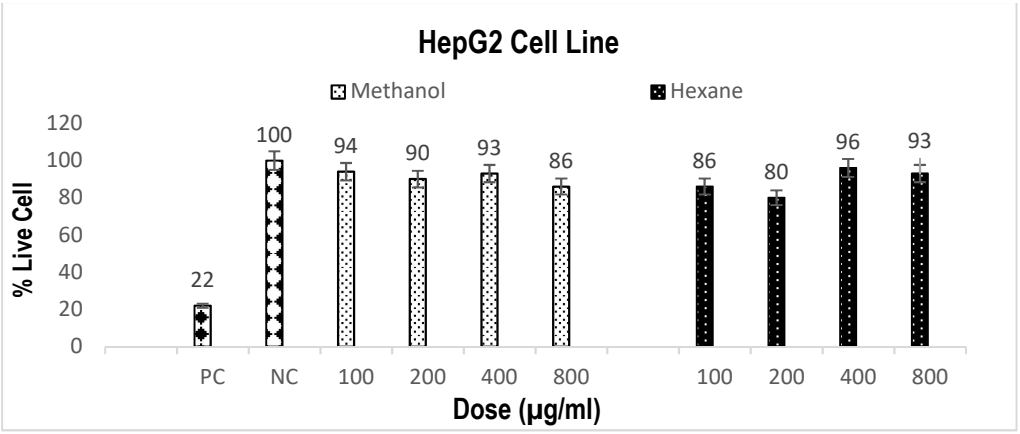

Figure 1. Cytotoxic activity of *E. vulgare* against HepG2 cell line.
*PC: Positive control: 100 µL Doxorubicin, NC: Negative control 100 µL RPMI

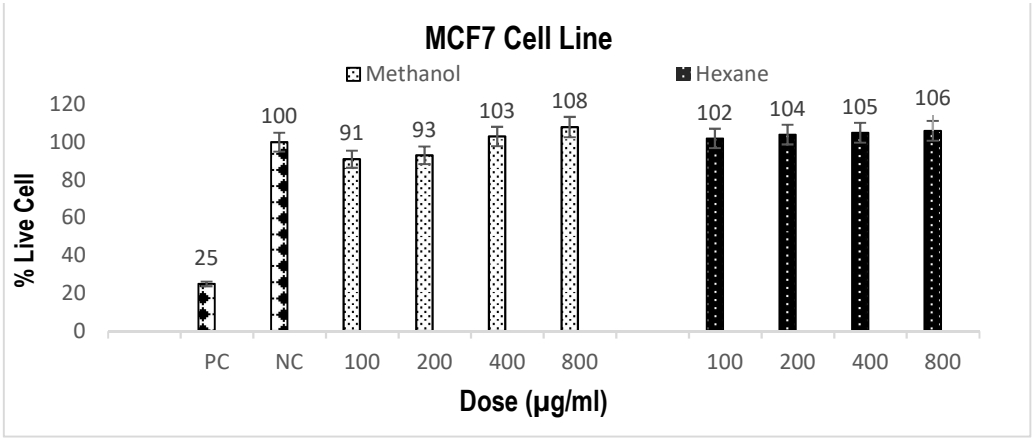

Figure 2. Cytotoxic activity of *E. vulgare* against MCF7 cell line.
*PC: Positive control: 100 µL Doxorubicin, NC: Negative control 100 µL RPMI

3.4. Antioxidant activity

Antioxidant activities of methanol and hexane extracts of *E. vulgare* seed were evaluated by DPPH method. According to the results shown in Table 3, the methanol extract of *E. vulgare* seed showed a percentage decrease of 4.5% at a concentration of 100 µg/mL, while the hexane extract showed a percentage decrease of 4.4% at a concentration of 100 µg/mL. Low antioxidant activity was observed at the concentrations tested and a dose-dependent increase was observed.

Table 3. Antioxidant activity results of extracts by DPPH radical scavenging capacity method.

Concentrations	12,5 µg/ml		25 µg/ml		50 µg/ml		100 µg/ml		Positive control (Ascorbic acid)
	Methanol	Hexane	Methanol	Hexane	Methanol	Hexane	Methanol	Hexane	
Samples	4.5±3.4	4.4±3.9	2.2±1.2	2.2±1.7	1.1±0.8	1.1±0.5	0.6±0.5	0.5±0.03	96,97

4. Discussion

There are some published papers including results on various activities of *E. vulgare*. In one of these studies, antibacterial and antitumor assays were performed to determine the biological activities of 16 different plants growing in Bolu province, including *E. vulgare*. Three different extracts were prepared for each plant: aqueous, ethanol and methanol. Antibacterial activity was evaluated on 10 different microorganisms (*Salmonella typhimurium, Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Enterobacter cloacae, Streptococcus pyogenes, Staphylococcus aureus and Staphylococcus epidermidis*) by disc diffusion method. The final concentration was considered to be 100 mg/mL. As a result of this evaluation, the methanolic extract of *E. vulgare* showed no antibacterial activity against any bacteria, while the ethanol extract showed the best antimicrobial activity on *S. epidermidis* with a zone diameter of 7.5 mm. Furthermore, the aqueous extract showed a zone diameter of 8.2 mm against *E. cloacae*. In this study, no antimicrobial activity was observed against the same microorganisms such as *E. coli* and *K. pneumaniae*, while in our study, it was observed that it formed a zone diameter against these microorganisms. This is thought to be due to the use of seed extracts instead of the above ground parts of *E. vulgare* and the different solvents used (Karakaş et al., 2012). Silver nanoparticles (AgNP) were produced by an ecological method using aqueous extract of *E. vulgare*. The antioxidant properties of these nanoparticles were evaluated by DPPH free radical scavenging assay and the IC_{50} value of AgNP was determined to be 14.7 ± 0.5 µg/mL. Furthermore, these nanoparticles were found to possess various biological activities such as anticancer and antioxidant, including methylene blue degradation (Gecer & Erenler, 2023). Phenol components of the extracts of the above-ground parts of *E. vulgare L*. and their antimicrobial and antioxidant effects were evaluated in different solvents including ethanol, acetone, chloroform, petroleum ether and ethyl acetate. Microdilution technique was used to determine their antimicrobial activity. Minimum inhibition concentration (MIC) and minimum bactericidal concentration (MBC) were determined with 20 different microorganisms including *E. coli, K. pneumaniae, S. aureus* and *C. albicans*. Antimicrobial activity results showed that *E. vulgare L.* extracts, especially chloroform and ethanol extracts, were superior to other extracts and some standard antibiotics (such as tetracycline and ketoconazole). Different tests were used to determine

the antioxidant properties of the plant extracts and the results showed that the ethanolic extract contained the most flavonoids and phenols, while the chloroform extract had the most effective antioxidant activity. Compared to our study, although similar methods and the same microorganisms were used for antimicrobial and antioxidant activity, the above ground parts of *E. vulgare* were used in the aforementioned study. In addition, the fact that the extracts are not the same reveals that the studies are not similar (Bošković, 2022). A detailed phytochemical analysis was carried out by obtaining both aqueous and ethanolic extracts from the above ground parts of *E. vulgare* growing in Ukraine. Within the scope of these analyses, qualitative evaluation of the active components of the plant was carried out and the amount of flavonoid components was quantitatively determined by a special method, spectrophotometric technique with aluminium chloride. Results showed that the total flavonoid concentration in the extract was 2.59% and the maximum yield obtained from this extraction procedure was 16% (Kapusterynska et al., 2020). Antioxidant properties of the methanol based extract of *E. vulgare* were investigated by DPPH methodology, focusing on phenolic and flavonoid components. High Performance Liquid Chromatography (HPLC) technology was preferred for the identification of phenolic and flavonoid substances. Analysis revealed that the antioxidant activity potential of the methanol extract of *E. vulgare* is due to its high phenolic and flavonoid content. Analysis of phenolic compounds by HPLC showed that substances such as gallic acid, benzoic acid and isoferulic acid were dominant. In flavonoids, quercetin and naringin were prominent. Based on these data, the polyphenolic components in *E. vulgare* extract were found to contribute positively to the maintenance of ideal weight, lipid balance and liver function indicators such as Alanine (ALT) and Aspartate (AST) transaminases. Recent analyses suggest that the richness of phenolic and flavonoid compounds has positive effects on blood and lipid balance and could potentially support heart and liver function (Alsanie et al., 2018). In vitro activity of *E. vulgare* L., *Lathyrus pratensis L., Glaucium leiocarpum Bois.* and *Ulmus minor Mill.* subsp. minor extracts against *Helicobacter pylori* and some mycobacter species were investigated. Effects against *Helicobacter pylori* and some mycobacter species were evaluated under in vitro conditions. Plants were dried, ground and extracted with 70% ethanol by maceration method. Obtained liquid extract was concentrated in vacuum and dissolved in water-ethanol (90:10) mixture. This solution was treated separately with dichloromethane and ethyl acetate to obtain sub extracts. Antibacterial activity of these extracts was investigated employing a modified Clinical and Laboratory Standards Institute technique (CLSI) against *Helicobacter pylori* ATCC 43504, *Mycolicibacterium smegmatis* ATCC 14468, and *Mycobacterium avium* ATCC 25291 liquid microdilution test. Stock solutions of the extracts dimethylsulfoxide (DMSO) were found to be effective against *U. minor* was found to be effective against *H. pylori* with an MIC (Minimum Inhibition Concentration) value of 250 µg/mL. However, other extracts did not show a significant efficacy at a concentration of 2000 µg/mL. According to the results of the study, the ethylacetate extract of *U. minor* can be considered as a potential treatment for *H. pylori* infections (Karadağ et al., 2020). Morphometric and phytochemical characteristics of seeds from three different populations of *E. vulgare* L. were analysed for comparative purposes. Two populations (labelled MZ and MC) are under the influence of Zn Pb mineral smelting and post processing waste deposits, so these two populations were termed "metallic populations" and the third population was collected from an area free of metal contamination (NM). In addition to the length, width, weight and surface measurements of these seeds, the germination capacity and viability values of the seeds of each population were determined and the amount of heavy metals, seed oil and secondary metabolite levels contained in the seeds were measured. According to the data obtained Upon completion of the investigation, it was noted that the seeds from metallic populations were characterised by smaller size and lower germination capacity, but the seeds of MC population showed the highest viability value. Seeds of the MC population were also characterised by the lowest levels of allantoin and rosmarinic acid and contained a higher seed oil content. Seeds of the MC population were found to be composed of 60% saturated and 45% monounsaturated fatty acids, but the proportion of polyunsaturated fatty acids was 16% lower than in the NM population (Dresler et al., 2017).

In previous studies, the above-ground parts of *E. vulgare* (such as leaves and stems) were used. In our study, 4 different microorganisms were used to determine the antibacterial activity of *E. vulgare* seeds and *C. albicans* was used to evaluate the antifungal effect. In order to detect the cell viability test of *E. vulgare*, cytotoxic effect of *E. vulgare* was examined using HepG2 and MCF7 cell lines. Antioxidant effect was analyzed by DPPH method. As a result, it was observed that *E. vulgare* showed the best inhibitory effect on *S. auerus* in terms of antimicrobial activity and did not show a successful effect against neither HepG2 nor MCF7 cell lines in terms of cytotoxicity. In the antioxidant determination of *E. vulgare*; it was seen that the effect increased with increasing concentration. This is in parallel with other studies on *E. vulgare*.

5. Conclusions

At present day, it is important to evaluate the cytotoxic, antioxidant and antimicrobial activities of plant extracts. In this study, anticancer, antioxidant and antimicrobial activities of *E. vulgare* seed were investigated. When the cytotoxic activities in different solvents (methanol and hexane) were compared, it was observed that the results of both solvents were parallel in antioxidant activity, which did not give a positive result. According to the antimicrobial activity values, the strongest inhibitory effect was observed on *S. aureus*, while the weakest inhibitory effect was observed on *C. albicans*. The findings indicate that herbal extracts and the compounds they contain in different combinations have the potential to be used as adjuvants in cancer treatment. However, in order to fully understand the true effects of these extracts, their biological activities need to be studied more extensively with detailed in vitro and in vivo experiments on animal models. Such comprehensive studies will reveal the true potential and safety of plant extracts in cancer treatment and guide their therapeutic application on a scientific basis.

Conflicts of Interests
Authors declare that there is no conflict of interests.

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Statement contribution of the authors
Dilek Arslan Ateşşahin: Project management, Conceptualization, Resources, Writing and editing. Lütfiye Kadıoğlu Dalkılıç: Formal analysis, review, and editing. Yasemin Özeren: Data curation. Semih Dalkılıç: Verification and editing. Kübra Çakmak: Data curation, Writing, editing. Tuğrul Arslan Çiçek: Visualization.

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