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Evaluation of cytotoxic, antimicrobial, and antioxidant activities of *Echium italicum* L. in MCF-7 and HepG2 cell lines

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ABSTRACT

The use of plants for medicinal purposes from past to present continues as traditional treatments. One of these plants, *Echium* species, is known for its high phytochemical content and its use as a sedative or in colds. In this study, the antibacterial, antioxidant and anticancer properties of *Echium italicum* L. were investigated. Its anticancer effect was tested in HepG2 (liver cancer) and MCF-7 (breast cancer) cell lines. Its antimicrobial effect was also investigated in *Candida albicans*, *Klebsiella pneumoniae*, *Escherichia coli*, *Bacillus megaterium* and *Staphylococcus a*ureus. The results showed that the highest cytotoxic effect was observed in the HepG2 cell line with 23% dead cell count. This suggests that the plant is especially effective in liver cancer cell lines. It was found that the extract prepared in hexane had the largest inhibition zone diameter (15 mm) against *C. albicans* in terms of antibacterial activity. The highest DPPH radical scavenging activity was observed in methanol extract with 37.6%. Considering the IC_{50} value in DPPH, hexane extract exhibited better activity than methanol extract (IC_{50} = 20.7 µg/ml). The data obtained from the study indicate that *E. italicum* has cytotoxic, antimicrobial and antioxidant effects. It is thought that the present study may contribute to the development and design of new anticancer and antimicrobial drugs, especially in the field of cancer research and pharmacology. In addition, it was concluded that the studies on the medical potential of *E. italicum* should be expanded and more comprehensive studies should be conducted.

1. Introduction

Plants have been widely consumed as food, medicine, spices and cosmetic products since the beginning of human history, and their most important feature is their use for therapeutic purposes. Most of the plants used for therapeutic purposes are collected from nature. This form of treatment using plants is used in many countries, especially in underdeveloped countries, and is known as traditional treatment, complementary treatment or natural treatment (Acıbuca & Budak, 2018; Petrovska, 2012; Shah et al., 2023). Plant-derived nanoparticles are now of great importance in the treatment of bacterial infections, especially multidrug-resistant pathogens (Waseem et al., 2023) and cancer (Naveed et al., 2024). This study examines *Echium italicum* L., which belongs to the Boraginacea family. This family includes more than 2000 species and 100 genera, and is especially common in temperate and tropical regions (Uysal et al., 2021; Xu & Deng, 2017). There are nine *Echium* species in Turkey. These are *E. angustifolium*, *E. arenarium*, *E. italicum*, *E. glomeratum*, *E. oriantale*, *E. parviflorum*, *E. plantagineum*, *E. russicum*, and *E. vulgare* (Eruygur et al., 2016).

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Echium species are known to be used as a cold, sedative, headache and stomach ache, wound healer, expectorant and laxative (Ahvazi et al., 2012; Karakaş et al., 2012; Nićiforović et al., 2010). *Echium* species stand out with their rich phytochemical content and contain various compounds such as fatty acids, amino acids, essential oils, pyrrolizidine alkaloids, naphthoquinones, flavonoids and anthocyanins. From the roots of *Echium* plants, a large number of shikonin derivatives have been identified. Shikonin, acetyl shikonin, deoxy shikonin, β, β-dimethylacrylshikonin, isobutrylshikonin, isovalerylshikonin, and 2-methyl butrylshikonin are some of the derivatives among naphthoquinones. Many researchers are interested in shikonin because of its many potential pharmacological properties, including antibacterial, anticancer, wound healing, and antioxidant qualities (Albreht et al., 2009; Eruygur, 2018). The ability of *E. italicum* callus to produce shikonin was also established, and it was shown that this plant could be a new source for the industrial production of shikonin and its derivatives (Zare et al., 2011).

E. italicum has an important place in folk medicine practices. In particular, *E. italicum* tea has been reported to have diuretic, sweet and soothing properties. Crushed leaves are used to ripen abscesses, relieve rheumatic pains and dissolve wound clots. In this application, the leaves are mixed with flour and turned into powder and applied externally. In addition, an ointment prepared by peeling the root bark of *E. italicum* and frying it in a pan with butter is used externally in wound healing. Decoctions prepared from *E. italicum* have various medicinal properties and offer various therapeutic benefits. These decoctions act as a purgative, sudorific and diuretic. They are also known for their emollient properties that accelerate the healing process. They have also been reported to be successful in treating various diseases, including respiratory tract infections. For this reason, *E. italicum* decoctions are considered a versatile healing source among the public (Eruygur et al., 2016). The seed oil content of *E. italicum* from Turkey was found to be rich in the unusual fatty acids stearidonic, gamma-linolenic acid and phytosterols and has high product potential as a source of complementary phytochemicals in the cosmetic and nutraceutical industries (Özcan & Süzerer, 2020).

The aim of this study was to evaluate the cytotoxic activity of *E. italicum* on human breast cancer (MCF-7) and human liver cancer (HepG2) cell lines and to determine its antimicrobial and antioxidant activity.

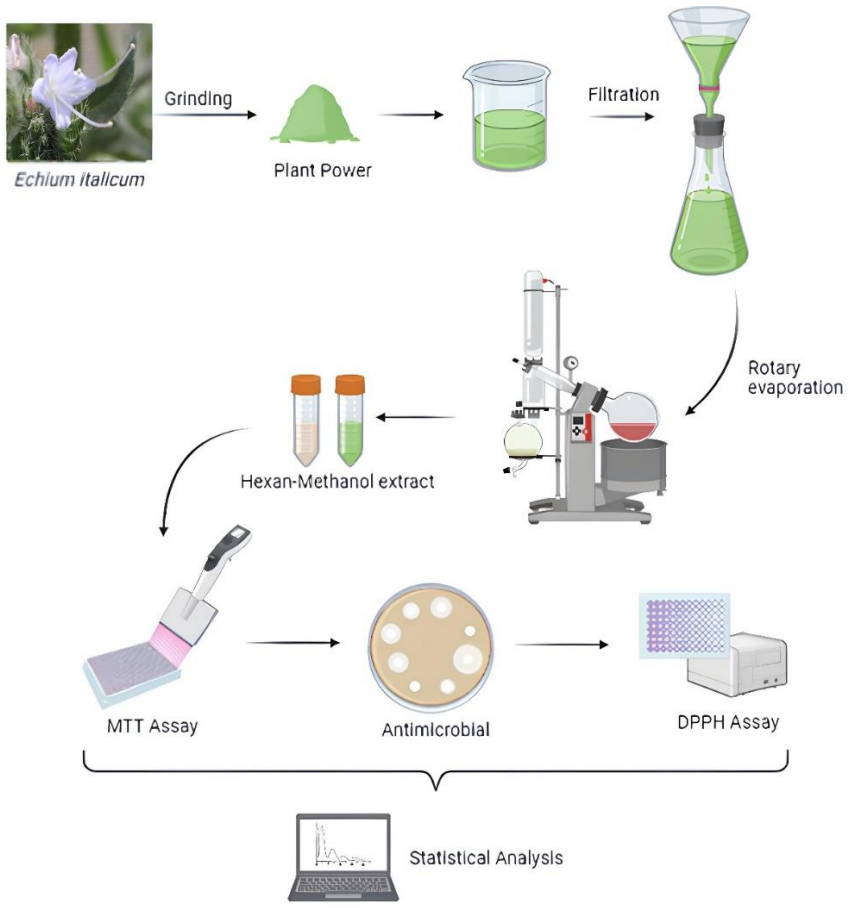

Figure 1. The methods applied to the *E. italicum* plant
Anticancer, antimicrobial, and antioxidant activities were evaluated in methanol and hexane

2. Materials and methods

2.1. Experimental desing

E. italicum belonging to the Boraginaceae family was collected and an experimental setup was established to evaluate its cytotoxic, antimicrobial and antioxidant activities. Firstly, the collected plants were dried and extracts were prepared in hexane and methanol. MCF-7 and HepG2 cells were treated with these extracts and their effects on cell viability were measured by MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide] assay. Additionally, the effects of the extracts on various bacterial and fungal species were studied to determine their antimicrobial effects. DPPH (2,2-Diphenyl-1-picrylhydrazyl) free radical scavenging assay

was applied to determine antioxidant activity. The obtained data were statistically analyzed to provide information on the potential effects of E. italicum. (Figure 1).

2.2. Materials

2.2.1. Procurement of material

E. italicum was collected from Baskil district of Elazığ province in June and July 2023. Taxonomic diagnosis and confirmation of the species were carried out by Assoc. Prof. Dr. Gülden Doğan in the Biology Department of Fırat University, Faculty of Science. Later, the specimen of *E. italicum* was preserved in the Fırat University Herbarium (FUH-8414).

2.2.2. Cell lines and microorganisms used in the experiment

Cell lines (MCF 7 and HepG2) were provided by the laboratory of Assoc. Prof. Dr. Semih Dalkılıç, faculty member of the Molecular Biology and Genetics Program of the Biology Department of Fırat University. Gram-negative (-) *Escherichia coli* ATCC 25322, gram-positive (+) *Staphylococcus aureus* ATCC 25923, *Bacillus megaterium* DSM32 bacteria and *Candida albicans* FMC17 fungus were provided by the central laboratory of Fethi Sekin City Hospital.

2.3. Methods

2.3.1. Extract preparation

The flowers, stems and leaves of *E. italicum* were thoroughly washed, separated and shade dried in the laboratory. After the dried plant was pulverized, 1 g was weighed and the extraction was carried out in 10 ml of methanol and hexane separately. The extracts were incubated in a shaking oven (Nücleon NCI55) at 37 °C for 72 h. After filtering through Whatman No. 1 filter paper, the extracts were evaporated in a rotary evaporator at 40 °C for four h. 10 ml of dimethylsulfoxide (DMSO) was added to each extract and stored at +4 °C until needed. The yield (%) for the obtained extract was determined (Arslan Ateşşahin et al., 2023; Kaptaner İğci & Aytac, 2020).

$$Percentage\ efficiency\ (w/w) = \frac{Weight\ of\ the\ extract\ (g)}{Weight\ of\ dry\ plant\ material\ before\ the\ extraction\ process\ (g)} x\ 100$$

2.3.2. Cell culture

HepG2 and MCF 7 cells were cultured in 75 cm² vials containing RPMI (1% Penicillin-Streptomycin, 10% Fetal Bovine Serum, 25 mM L-Glutamine) at 37 °C and in a 5% CO_2 atmosphere.

2.3.3. Cytotoxic activity

MTT is a tetrazolium salt used only to detect living cells. This test is used to measure the metabolic activity of cells and is often used in cell proliferation and cell viability assessments. The MTT test is based on the conversion of MTT metabolized by cells into a product called formazan. The formazan formed is detected by measuring its absorbance at an appropriate wavelength in a scanning multiwell spectrophotometer, such as an ELISA reader. This method is a rapid and sensitive colorimetric test method widely used to assess the viability and metabolic activity of cells (Mosmann, 1983).

In 75 cm² vials, 90% of the confluent cells were pelleted, lysed with RPMI and counted with the Countess II instrument. Then, 5 × 10^3 cells per well were seeded in 96-well plates and incubated overnight at 37 °C (5% CO_2). The medium was removed from the wells and four different concentrations of two *E. italicum* extracts (100, 200, 400 and 800 µg/µl) were added. RPMI was used as negative control and 2.5 µg/ml doxorubicin (DOXO) as positive control. 10 µl of MTT (MCE MedChem Cell Counting Kit-8) solution was added to the plates, incubated for 72 h at 37 °C (5% CO_2) in the dark. The color change was measured with an ELISA (Enzyme Linked Immuno Sorbent Assay) microplate reader at a wavelength of 450 nm. The following formula was used to calculate toxicity:

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

2.3.4. Antimicrobial activity

E. italicum methanol and hexane extracts were tested for antibacterial activity using agar well diffusion method. Agar well diffusion method, a popular technique, was used to evaluate the antibacterial activity of the studied plant and chemical components (Balouiri et al., 2016).

Mueller hinton agar (MHA) (Merck Lot: VM779137) was used for the agar well diffusion method. Microorganisms were grown on Sabouraud dextrose agar (SDA) and Nutrient broth (Biolife Lot: HE2602). Bacterial strains were grown at 37 ± 0.1 °C for 24 h and diluted to a McFarland setting of 0.5 (10^8 microorganisms/ml). Fungal strain was inoculated into petri dishes containing SDA and incubated for 48 h (31 °C). Yeast colonies were suspended in 0.9% sodium chloride (saline) solution and incubated at 45 °C to reach a concentration of 2 × 10^7 cells/ml. The reconstituted and autoclaved sterilized (121 °C for 15 min) MHA was distributed in 15-20 ml volumes into petri dishes and allowed to cool. 100 µl of microorganism suspension was added to the solidified MHA and spread evenly on the agar using a drigalski spatula. Wells of the desired diameter were obtained with an agar borer (cork borer). Four concentrations of extracts (25, 50, 75 and 100 mg/ml) were prepared and mixed under aseptic conditions at 100 µl per well. Incubation for bacterial and fungal inoculated plates was 24 h (37 ± 0.1 °C) and 48 h (25 ± 0.1 °C), respectively. A standard disk containing 100% DMSO (Merc-Lot K51154243 948) was used as a negative control and 2 µg clindamycin (Lot 171127A) was used as a positive control. Inhibition diameters were measured after incubation (Aytar et al., 2019; Dalkılıç et al., 2023).

2.3.5. Antioxidant activity

Methanol and hexane extracts of *E. italicum* were tested for antioxidant activity using the DPPH radical scavenging capacity technique (Cuendet et al., 1997; Kirby & Schmidt, 1997). 0.25 mM DPPH radical solution (0.5 ml) was dissolved in methanol and serially diluted at 50 mg/ml concentrations of the extracts separately.

In the test, positive control of 100% ascorbic acid and negative control of 100% methanol were used. After the mixture was shaken well and left in the dark for half an hour, the absorbance at 517 nm was calculated. The following formula was used to determine the DPPH radical scavenging capacity (Dalkılıç et al., 2023):

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

2.3.6. Statistical analysis

All experiments were performed in triplicate independently. Standard deviations of antimicrobial data were calculated using GraphPad Prism 5.03 (GraphPad Software, Inc.; USA) software. The anticancer

results were calculated by one-way ANOVA using SPSS 22 for Windows, and the *p*-value was found to be greater than 0.05, indicating no statistically significant difference. According to these results, the *p* value of this plant was not found to be significant.

3. Results and discussion

3.1. Extract efficiency

The percentage efficiency of the extracts of *E. italicum* prepared in hexane and methanol was calculated (Table 1). According to the results obtained, the yield percentage of the prepared extract in hexane was found to be 0.34% and in methanol was found to be 3.32%.

Table 1. Extract results for percent efficiency*

Extracts	Dry leaf material before extraction (g)	Extract weight after extraction (g)	Yield of the extract (%)
Hexane	1	0.34	34
Methanol	1	0.33	33

*No loss of percentage efficiency of the extracts

3.2. Cytotoxic activity

When the cytotoxic activity of *E. italicum* was examined, it was found that the number of dead cells in the HepG2 cell line was determined as 23% at the highest concentration of methanol extract, 800 µg/ml. When the hexane extract was examined, the best cytotoxic activity was found to be 18% at the concentration of 400 µg/ml (Figure 2).

When the results in MCF-7 cells are examined in Figure 3, it was seen that 9% of the cells died at the lowest concentration of methanol extract (100 µg/ml). Hexane extract showed 20% cytotoxic activity at 800 µg/ml. The half maximum inhibitory concentration (IC_{50}) values of this plant in the indicated cell lines were calculated. The IC_{50} value expresses the concentration required to kill 50% of the cells, and the lower this value, the stronger the cytotoxic effect of the compound. According to the calculated IC_{50} values, the value of methanol extract was found to be 400 µg/ml and the value of hexane was found to be 291.8 µg/ml in the HepG2 cell line. This indicates that hexane extract may be more effective at lower concentrations than methanol extract in HepG2 cell line. In MCF-7 cell line, IC_{50} value of methanol extract was 202.2 µg/ml, while IC_{50} value of hexane extract was 853 µg/ml. This result suggests that methanol extract has a much more effective cytotoxic effect than hexane extract in MCF-7 cells. In general, the sensitivity of different cell lines to different extracts may be related to the biological properties of cells and their metabolic responses to chemical compounds (Table 2).

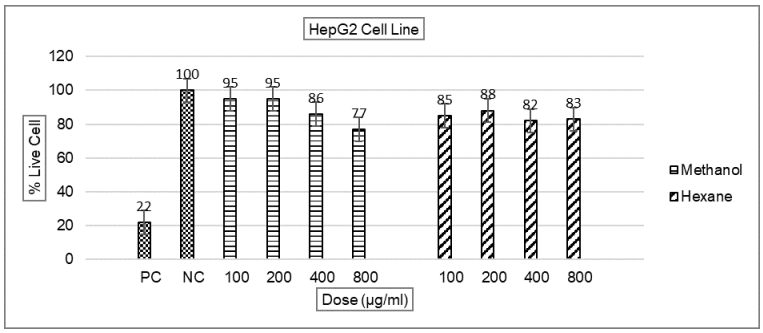

Figure 2. Results of the cytotoxic effect on the HepG2 cell line*

*PC = Positive control (DOXO 2.5 µg/ml), NC = Negative control (untreated cells), Cell lines: HepG2 (human hepatocellular carcinoma), Concentration: 100-200-400-800 µg/ml

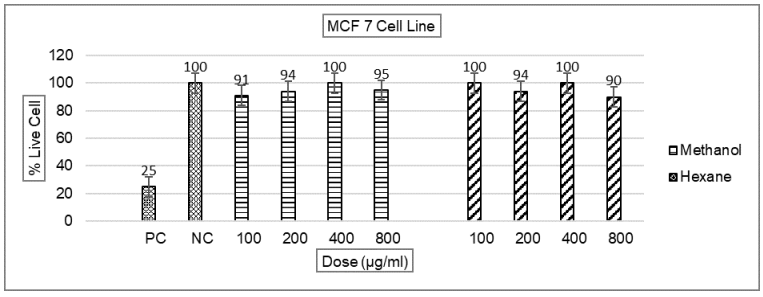

Figure 3. Results of the cytotoxic effect on the MCF-7 cell line*

*PC = Positive control (DOXO 2.5 µg/ml), NC = Negative control (untreated cells), Cell lines: MCF 7 (human breast cancer), Concentration: 100-200-400-800 µg/ml

In a study by Boškovic et al. (2017), ethyl acetate, chloroform, ethanol, acetone and petroleum ether extracts were prepared from *E. italicum*. DPPH assay was utilized to determine the antioxidant effects and the MTT method was used to determine the cytotoxic activity. Cytotoxic activity was tested in human cell line rhabdomyosarcoma RD, human laryngeal carcinoma Hep-2C and mouse tumor fibroblast L2OB cell lines. Ethanol and acetone extracts of the plant showed the best antioxidant activity. Cytotoxic activity (IC_{50}) of the

extracts ranged from 87.30 ± 4.09 µg/ml to 172.52 ± 2.44 µg/ml. The best cytotoxic activity was found to be 87.30 ± 4.09 µg/ml and 91.56 ± 2.31 µg/ml in L2OB cell line in the presence of chloroform and acetone extracts, respectively (Boškovic et al., 2017).

Table 2. Half inhibition (IC_{50}) value of cytotoxic activity on cells

Cell lines	Methanol	Hexane
HepG2	400 µg/ml	291.8 µg/ml
MCF-7	202.2 µg/ml	853 µg/ml

In another study by the same research team, acetone, ethanol, chloroform and ethyl acetate extracts of *Anchusa officinalis* L., *E. vulgare* and *E. italicum* were comparatively studied (Boskovic et al., 2021). Cytotoxic activities of the extracts were evaluated in Hep-2C, RD and L2OB cell lines using MTT assay. All the tested plants were observed to have inhibitory activity on the indicated cancer cell lines. *E. italicum* extract prepared with chloroform was found to have the highest cytotoxic effect on L20B cell line with an IC_{50} value of 87.30 µg/ml.

According to MTT results, the best IC_{50} value in the methanol extract used in MCF-7 was found to be 202.2 µg/ml. This shows that E. italicum exhibits better cytotoxic activity in the cell lines used in the study, especially the L2OB cell line. The activity of ethyl acetate, dichloromethane and hexane extracts of the flower of Echium amoenum, a species belonging to the Boraginaceae family, on the J774.1A macrophage cell line was analyzed by the MTT method (Naseri et al., 2018). In the study in question, where concentrations of 1, 10, 25, 50, 100 and 200 µg/ml were examined, it was observed that, similar to the current study, there was no significant cytotoxic activity between 1-100 µg/ml, and the best effect was found at 200 µg/ml. In another study conducted by our research group, MTT test was used to evaluate the cytotoxic effects of *E. vulgare* methanol and hexane extracts on MCF-7 and HepG2 cell lines. The best result was observed in hexane extract with 20% cytotoxic effect on HepG2 cell line (Arslan Ateşşahin et al., 2023). The highest cytotoxic effect of *E. italicum* on HepG2 cell line was observed in methanol extract with 23%.

3.3. Antimicrobial effect

The antibacterial effects of methanol and hexane extracts of *E. italicum* on five different microorganisms were tested and the findings are given in Table 3. Inhibitory zone diameter (mm) measurements were used to evaluate the antibacterial activity and DMSO was used as a negative control. In addition, the obtained data were compared with clindamycin used as a control group.

Table 3. Antimicrobial effect of *E. italicum* extract*

Microorganisms	Concentrations (mg/ml) and zone diameters (mm)								
	E. italicum methanol extract				*E. italicum* hexane extract				Clindamycin
	25	50	75	100	25	50	75	100	(2 µg)
B. megaterium	10 ± 1	11	11	12 ± 1	9 ± 1.5	10 ± 0.5	11 ± 0.5	12 ± 1.5	22 ± 0.6
E. coli	10 ± 2	12	12	14 ± 2	11 ± 1.7	13 ± 0.2	13 ± 0.2	14 ± 1.2	20 ± 1.4
S. aureus	11 ± 1.5	12 ± 0.5	13 ± 0.5	14 ± 1.5	11 ± 0.7	11 ± 0.7	12 ± 0.2	13 ± 1.2	23 ± 1.6
C. albicans	11 ± 1.2	12 ± 0.25	12 ± 0.2	14 ± 1.7	14 ± 0.7	15 ± 0.2	15 ± 0.2	15 ± 0.2	20 ± 1.4
K. pneumoniae	10 ± 2.5	12 ± 0.5	14 ± 1.5	14 ± 1.5	10 ± 1.5	11 ± 0.5	12 ± 0.5	13 ± 1.5	22 ± 0.6

*All experiments were repeated three times independently. Means are shown together with standard deviations. Standard deviations were computed with GraphPad Prism 5.03 (GraphPad Software, Inc.; USA) software.

The results showed that the methanol extract of *E. italicum* exhibited the highest zone diameter (14 ± 1.5 mm) at the highest concentration of 100 mg/ml and the lowest zone diameter (10 ± 1 mm) at the lowest concentration of 25 mg/ml. This situation was found to be the same for hexane extract. The lowest zone diameter (9 ± 1.5 mm) in hexane extract was observed against *B. megaterium* at the lowest concentration (25 mg/ml). The highest inhibitory activity was found against *C. albicans* with 15 ± 0.2 mm at 50, 75 and 100 mg/ml hexane extract concentrations (Figure 4).

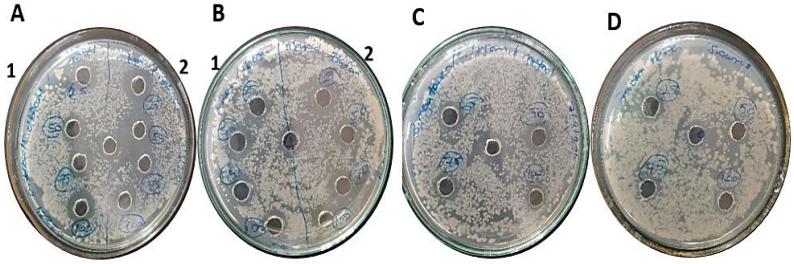

Figure 4. Inhibition zones (mm) of *E. italicum* against *C. albicans* with methanol (A1) and hexane (A2) solvents, against *E. coli* with hexane (B1) and methanol (B2) solvents, against *B. megaterium* with methanol (C) and against *S. aureus* with hexane solvents

In a study conducted by Morteza-Semnani et al. (2009), essential oil was isolated from E. italicum obtained from the northern region of Iran by hydrodistillation. Twenty-two compounds were identified and the main components of this oil were hexadecanol (27.1%) and pulegone (8.8%). Disk fusion method was used to test the antibacterial activity of this oil against the following microorganisms: *B. subtilis*, *S. aureus*, *E. coli*, *Salmonella typhi*, *Pseudomonas aeruginosa*, *Aspergillus niger* and *C. albicans*. The antimicrobial activity of the essential oil tested at concentrations of 250, 500, 1000, 2000, 4000 and 8000 µg/disc was found to increase with concentration.

In parallel with these results, in the present study, it was observed that the antimicrobial effect of *E. italicum* extract increased with increasing concentration (25, 50, 75, and 100 mg/ml). The antimicrobial effect of *E. amoenum* (Boraginaceae) flower collected from Karaj city of Iran was determined by disk diffusion method (Shariatifar et al., 2016). Ethanol extracts of the plant were obtained with 100% ethanol and distilled water, respectively. Both aqueous and ethanol extracts (AEs and EEs) showed antibacterial activity against both gram-positive and gram-negative bacterial strains. The best antimicrobial effect of EEs was found against *S. aureus* (inhibition zone diameter: 11.5 ± 0.87 mm). The highest zone diameters were found against *Listera monocytogenes* (13 ± 0.82 mm) and *Yersinia enterocolitica* (13.5 ± 1.04 mm). The inhibition zone was

found to be 11 ± 0.85 mm and 12 ± 1.21 mm against *S. aureus* and *E. coli*, respectively.

In the present study, the highest zone diameters obtained against S. *aureus* and *E. coli* were found to be 14 ± 2 mm and 14 ± 1.5 mm in the presence of methanol extracts, respectively. When compared with these results, it was determined that the highest concentrations of extracts prepared in methanol (100 mg/ml) showed better antimicrobial activity. In a study conducted by Fazilati and Dousti (2019), different parts of *E. italicum* showed antifungal activity against *C. albicans* in aqueous, *n*-hexane and methanol extracts. The mean growth inhibitory diameter of methanol and *n*-hexane extract at a dose of 5 mg/ml was greater than the diameter of the antibiotic nystatin for *C. albicans* ($p < 0.05$). The methanol extract of plant root had the lowest MIC and MBC for *C. albicans* with a value of 15.62 µg/µl.

3.4. Antioxidant activity

The antioxidant activity of *E. italicum* methanol and hexane extracts was evaluated by calculating the percentage reduction in DPPH radical at various doses. Figure 5 shows antioxidant activity and the reducing power in hexane extract was found to be between 21.1% ±0.06 and 36.9%. The results in methanol extract were found to be between 17.4% ± 1.85 and 37.6% ± 2, and the best reducing power was found to be 37.6% ± 2 in 50 mg/ml methanol extract. Table 4 shows the IC_{50} values of antioxidant activities of hexane and methanol extracts based on DPPH test. According to the results in the table, the IC_{50} value of the hexane extract was found to be 63.3 µg/ml and the IC_{50} value of the methanol extract was found to be 20.7 µg/ml. Considering the IC_{50} value, methanol extract showed better activity than hexane extract. This indicates that methanol extract showed much stronger antioxidant activity than hexane extract in neutralizing DPPH radicals. The low IC_{50} value of the methanol extract suggests that this extract contains compounds richer in antioxidant properties or neutralizes radicals with a more effective mechanism. In parallel with our study, it was observed that *E. italicum* extracts with high total phenolic content showed higher antioxidant activities compared to E. amoenum extracts with low total phenolic content (Abbaszadeh et al., 2013). The total antioxidant status of *E. italicum* was compared with many plants and revealed to be higher than *Scorzonera papposa*, *Adiantum calocephalum*, *Mentha longifolia* subsp. *longifolia* and *Adiantum capillus-veneris*, and lower than *Salvia absconditiflora*, *Thymbra spicata* and *Marrubium globosum*. In addition, analysis of the antioxidant and oxidant status of *E. italicum* samples collected from various parts of Turkey revealed that antioxidant and oxidant values varied according to the region. *E. italicum* was also shown to have the potential to be a natural antioxidant source (Uysal et al., 2021).

Table 4. IC_{50} values of antioxidant activity test

DPPH IC_{50}	IC_{50} value (µg/ml)
Hexane	63.3
Methanol	20.7

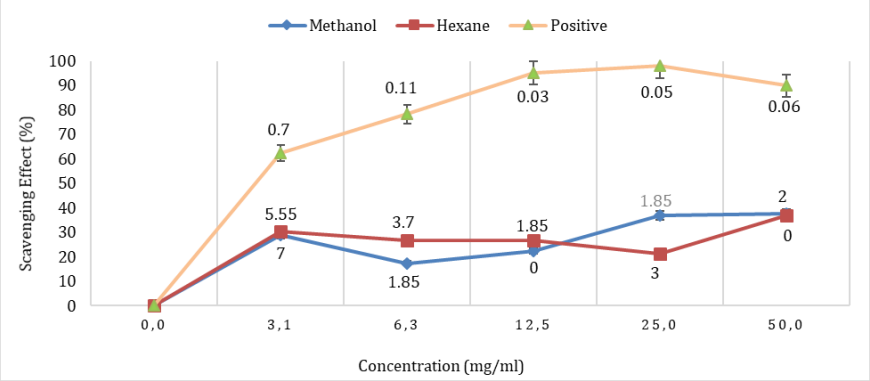

Figure 5. Percent inhibition of DPPH free radical scavenging activity*

Percentage change of DPPH radical scavenging activity of methanol and hexane extracts of *E. italicum*. *Positive control: Ascorbic acid, Negative control: Methanol

In a study by Bekhradnia and Ebrahimzadeh (2016), three different extracts were used to study the antioxidant activity of leaves: polyphenol (PP), ultrasonic (US) and alkaloid (AK) fractions. Compared to other extracts, US extract showed greater activity in scavenging DPPH radicals despite containing less total phenols. The IC_{50} value of ultrasonic extract was 78.0 ± 2.4 $\mu g.ml^{-1}$, followed by an alkaloid fraction with a value of 159.5 ± 6.9 $\mu g.ml^{-1}$. In the present study, hexane extract of *E. italicum* was found to have maximum DPPH radical scavenging activity (IC_{50} value: 20.7 µg/ml). The DPPH radical scavenging activity of methanol solution (IC_{50}) was 60.3 µg/ml. Therefore, it can be said that both hexane and methanol extracts of *E. italicum* have higher and better antioxidant effects than *E. ammoneum*.

4. Conclusions

In the present study, the anticancer, antioxidant and antimicrobial effects of *E. italicum* collected from Elazığ Baskil region were evaluated. According to the anticancer activity results, methanol extract showed the best cytotoxic effect at the highest concentration on HepG2 cell line. In terms of antimicrobial effect, the strongest inhibitory effect was found against *C. albicans* and the lowest effect was found against *B. megaterium*. The results also revealed that *E. italicum* extracts prepared with both solvents showed antioxidant activity. With the data obtained from the study, it can be said that *E. italicum* has a high potential in terms of antimicrobial and antioxidant effects, but it showed partial effect against the cell lines tested in terms of anticancer properties. Despite having a promising potential in terms of antimicrobial and antioxidant properties, the extract in question showed low cytotoxic effect in the studied cell lines. Considering this situation, it is thought that *E. italicum* should be tested in different cell lines and its anticancer potential should be tested more comprehensively. Therefore, clinical studies are needed to fully investigate the therapeutic potential of *E.italicum*.

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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. On request, the associated author can provide more information.

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CRediT authorship contribution statement

Dilek Arslan Atessahin: Project management, Data curation, Auditing
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Supplementary File

None.

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