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Original Article

Phytochemical analysis and antioxidant, antimicrobial, cytotoxic activities of different solvent extracts of *Zygophyllum fabago* L.

Análise fitoquímica e atividades antioxidante, antimicrobiana e citotóxica de diferentes extratos solventes de *Zygophyllum fabago* L.

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Abstract

This study investigates the phytochemical composition, antioxidant, antimicrobial, and cytotoxic properties of six solvent extracts (ethanol, ethyl acetate, and hexane) obtained from the roots and aerial parts of *Zygophyllum fabago* L. growing in the arid and saline regions of southern Kazakhstan. Antioxidant potential was assessed using DPPH and ABTS radical scavenging assays, with Trolox as a reference standard. The minimum inhibitory concentration (MIC) of the extracts was determined using the broth microdilution method. The ethanol extract of aerial parts demonstrated the highest total phenolic content (128.29 ± 0.81 mg GAE/g) and the strongest antioxidant activity (DPPH SC_{50} = 42.57 ± 3.03 µg/mL), where SC_{50} represents the concentration required to scavenge 50% of free radicals. Ethyl acetate extracts exhibited significant antimicrobial activity, particularly against *E.coli* and *C.albicans* (MIC <9 µg/mL). Cytotoxicity was assessed using the MTT assay on NIH-3T3, K562, and Saos cell lines. The ethyl acetate extracts (both roots and aerial parts) were most effective against K562 leukemia cells, with IC_{50} values of 121.0 ± 2.5 µg/mL and 120.4 ± 3.1 µg/mL, respectively. These findings highlight the potential of *Z. fabago* extracts as natural antioxidants and antimicrobial agents.

Keywords: *Z. fabago*, phytochemicals, biological activities, DPPH, MIC, IC50.

Resumo

Este estudo investiga a composição fitoquímica, bem como as propriedades antioxidantes, antimicrobianas e citotóxicas de seis extratos obtidos com diferentes solventes (etanol, acetato de etila e hexano), a partir das raízes e partes aéreas de *Zygophyllum fabago* L., coletadas em regiões áridas e salinas do sul do Cazaquistão. O potencial antioxidante foi avaliado por meio dos ensaios de sequestro dos radicais DPPH e ABTS, utilizando Trolox como padrão de referência. A concentração inibitória mínima (CIM) dos extratos foi determinada pelo método de microdiluição em caldo. O extrato etanólico das partes aéreas apresentou o maior teor de compostos fenólicos totais (128,29 ± 0,81 mg EAG/g) e a mais potente atividade antioxidante (DPPH SC_{50} = 42,57 ± 3,03 µg/mL), sendo SC_{50} a concentração necessária para neutralizar 50% dos radicais livres. Os extratos em acetato de etila demonstraram atividade antimicrobiana significativa, especialmente contra *E.coli* e *C.albicans* (CIM < 9 µg/mL). A citotoxicidade foi avaliada pelo ensaio MTT em linhagens celulares NIH-3T3, K562 e Saos. Os extratos em acetato de etila – de raízes e partes aéreas – foram mais eficazes contra células leucêmicas K562, com valores de IC_{50} de 121,0 ± 2,5 µg/mL e 120,4 ± 3,1 µg/mL, respectivamente. Esses resultados destacam o potencial dos extratos de *Z. fabago* como agentes naturais antioxidantes e antimicrobianos.

Palavras-chave: *Z. fabago*, fitoquímicos, atividades biológicas, DPPH, CIM, CI_{50}.

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1. Introduction

Traditional medicine has relied on plants for therapeutic purposes for centuries (Yuan et al., 2016). Due to the lack of knowledge about the causes of diseases or which plants could be used as treatments, early medicinal practices were based solely on trial and error. Over time, the use of medicinal plants went beyond simple empirical observations and became supported by scientific understanding (Karunamoorthi et al., 2012). Expanding the list of promising medicinal plants is a key priority as the demand for them continues to increase every day (Petrovska, 2012). *Zygophyllum fabago* (*Zygophyllaceae*), also known as the "Syrian caper," is a medicinal plant traditionally used to treat bladder conditions, rheumatism, and skin diseases. It also exhibits anthelmintic, antisyphilitic, laxative, anti-inflammatory, and antimicrobial properties (Zaidi and Crow Junior, 2005). *Z. fabago* is a plant that has leaves that branch out as they grow. It typically reaches a height of 30-75 cm. The leaves are arranged opposite each other on the stem and have a small appendage at the tip. These leaves are fleshy in texture and vary in size. The flowers of the plant are white with orange markings at the base. The corolla is predominantly white, accented by an orange center, with petals that closely match the sepals in length, measuring 5 to 8 mm. The seeds of this plant are flattened and have a yellowish-gray tint (Grudzinskaya et al., 2014). *Z. fabago* thrives in salt marshes, sandy hills, and saline soils in southern and southeastern Kazakhstan (Gemejiyeva et al., 2022).

This study aims to evaluate the phytochemical composition, antioxidant, antimicrobial, and cytotoxic properties of ethanol, ethyl acetate and hexane extracts from the aerial and root parts of *Zygophyllum fabago* to assess their pharmacological potential and possible applications in medicine and industry. The selection of these solvents was based on their polarity (Zeng et al., 2011), allowing for the extraction of a broad range of phytochemicals, from highly polar phenolics (ethanol) to moderately polar flavonoids and tannins (ethyl acetate) and non-polar terpenoids and lipids (hexane) (Nawaz et al., 2020). Additionally, the study employs three distinct cell lines—NIH-3T3 (mouse embryonic fibroblast), K562 (chronic myeloid leukemia), and Saos (human osteosarcoma). These were chosen to assess both the general cytotoxicity of the extracts (NIH-3T3 as a normal cell model) and their potential anticancer effects (K562 and Saos as cancer cell models) (Jahanian-Najafabadi et al., 2019). The findings of this study contribute to understanding the pharmacological potential of *Z. fabago* and its possible applications in medicine and industry.

2. Experimental

2.1. Plant material

Z. fabago was collected in the Balkhash district of the Almaty region, Kazakhstan. GPS (Garmin eTrex22x) coordinates (45°05'19.5″ N and 75°00'46.5″ E). *Z. fabago* was collected in the morning during the period of active vegetation in June 2024. The raw plant material was collected in ecologically clean areas, and the species was identified and authenticated by the staff of the Institute of Botany and Phytointroduction (Almaty, Kazakhstan). To confirm the identification, part of the collected material was preserved and transferred for storage to the herbarium of the Institute of Botany and Phytointroduction, Almaty, Kazakhstan (voucher no: 05/337). The harvested plant was cleaned and dried in the dark at room temperature for at least ten days. The dried material was then processed into a uniform fine powder using an electric micro-grinding machine (110 V, 1400 rpm), ensuring the required consistency for further analysis.

2.2. Extraction of phytochemicals

The shade-dried plant material was macerated (cold extraction) with 70% ethanol in a ratio of 1:8 (w/v) at room temperature for 24 hours. The obtained extract was filtered using a rotary vacuum evaporator (*Buchi Rotavapor*, R-300, Switzerland) at an internal temperature not exceeding 40°C until dry residue. The same method was applied for ethyl acetate and hexane solvents. The % yields were calculated using the formula (Equation 1):

$$\%\ yield = \left(W_1 / W_2\right) / 100 \quad (1)$$

where:
W_1 is the weight of dried extract and W2 is the dry weight of the dried plant. A total of six different solvent extracts were prepared: ethanol, ethyl acetate, and hexane extracts from roots and aerial parts of *Z. fabago*. The solvents were selected based on their ability to extract a diverse range of phytochemicals (Luan et al., 2024).

2.3. Phytochemical and physicochemical screening test

Moisture content was determined by drying 1 g of powdered *Z. fabago* plant material to a constant mass at 100–105 °C, while total ash content was assessed by incinerating 2 g of material at 500–600 °C (Musinov and Tulagenova, 2016). Total tannins were quantified using an indigosulfonic acid-based titration method, where extracts from 3 g of *Z. fabago* were titrated with 0.02 M potassium permanganate until a golden-yellow endpoint was observed (Savarirajan et al., 2021). The tannin content was calculated as a percentage relative to the dry weight. Total saponin content was determined by maceration of 2 g of *Z. fabago* with a 3% acetone solution of nitric acid, followed by ethanol washing and precipitation with ammonia. The resultant precipitate was dissolved in water, and absorbance was measured at 258 nm using a UV-Vis spectrophotometer Total saponin content was determined by maceration of 2 g of *Z. fabago* with a 3% acetone solution of nitric acid, followed by ethanol washing and precipitation with ammonia. The resultant precipitate was dissolved in water, and absorbance was measured at 258 nm using a UV-Vis spectrophotometer (Parbuntari et al., 2018). Organic acids were quantified by boiling 10 g of plant material in distilled water, followed by titration with 0.1 M sodium hydroxide in the presence of

phenolphthalein, and expressed as a percentage relative to the dry weight (Geng et al., 2008). Total flavonoid content was determined using an aluminum chloride colorimetric assay. Extracts from 2 g of *Z. fabago* were refluxed with 90% ethanol containing 1% hydrochloric acid. A 2 mL aliquot of the extract was mixed with 1 mL of 1% aluminum chloride solution, and absorbance was measured at 430 nm after 20 minutes. Total flavonoid content was determined using an aluminum chloride colorimetric assay. Extracts from 2 g of *Z. fabago* were refluxed with 90% ethanol containing 1% hydrochloric acid. A 2 mL aliquot of the extract was mixed with 1 mL of 1% aluminum chloride solution, and absorbance was measured at 430 nm after 20 minutes. Flavonoid content was expressed as quercetin equivalents (mg QE/g dry weight) based on a standard calibration curve (Shaikh and Patil, 2020).

2.4. Total phenolic content determination

The total phenolic content of the extracts was estimated by the Folin-Ciocalteu method. The reaction mixture consists of 50 µL of 7% Na_2CO_3, 2.5 µL Folin-Ciocalteu reagent, 195 µL distilled water, and 2.5 µL extract. Absorption was measured at 765 nm following 30 minutes of incubation in the dark. The total phenolic content of the extract was calculated by comparison with a standard curve generated by analysing gallic acid (GAE) (Zhou et al., 2004).

2.5. Antioxidant Activity Assays. DPPH radical scavenging activity

The antioxidant activity of the extracts was evaluated using the DPPH assay, which measures the ability of the extracts to scavenge the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical (Alemu et al., 2024). Trolox was used as a positive control due to its well-established radical scavenging properties, while ethanol was used as the negative control. Sample stock solutions (1.0 mg/mL) were prepared and diluted to final concentrations of 500, 250, 125, 50, 25, and 10 µg/mL in ethanol. A reaction mixture was prepared by adding 1 mL of 0.3 mM DPPH ethanol solution to 2.5 mL of each sample solution. The mixtures were incubated at room temperature in the dark for 30 minutes, and the absorbance was measured at 518 nm using a UV-Vis spectrophotometer. The blank consisted of 1 mL of ethanol mixed with 2.5 mL of each plant extract, while the negative control consisted of 1 mL of DPPH solution combined with 2.5 mL of ethanol. Rutin (1 mM) was used as an additional positive control alongside Trolox to compare the radical scavenging efficiency of the extracts with a known flavonoid standard. The percentage antioxidant activity (AA) was calculated using the equation (Equation 2):

$$AA\% = 100 - \left[\begin{matrix} \left(Ab_{of\ sample} - Ab_{of\ blank} \right) \times \\ 100 \ / \ Ab_{of\ control} \end{matrix} \right] \quad (2)$$

where:
Ab sample is the absorbance of the test sample, *Ab blank* is the absorbance of the blank, and *Ab control* is the absorbance of the negative control (DPPH in ethanol). The SC_{50} value (half-maximal scavenging concentration) was determined by plotting the percentage of DPPH radical scavenging activity against the logarithm of extract concentration. The SC_{50} represents the concentration of extract required to scavenge 50% of DPPH radicals and was calculated using linear regression analysis based on triplicate measurements.

2.6. ABTS radical scavenging activity

ABTS was produced in this process by mixing 0.746 mM ABTS with 0.245 mM potassium persulfate, keeping it in the dark at room temperature for 24-48 hours before use. Trolox was used as a reference standard, and ethanol served as the negative control. The samples mixed with diluted ABTS solution (1/10) and ethanol. After a 6-minute reaction, the absorbance at 734 nm was measured, and then the inhibition percentage was calculated by the following equation (Equation 3):

$$\%\ Inhibition = \left[\begin{matrix} A_{blank} - \\ A_{sample} \ / \ A_{blank} \end{matrix} \right] x\ 100 \quad (3)$$

and the results were expressed as mg Trolox equivalents per gram of extract (Gulçin and Alwasel, 2023).

2.7. Determination of the antimicrobial activity of ethanol, ethyl acetate, and hexane extracts of Z. fabago

The antimicrobial activity of the extracts was investigated against five microbial strains, including two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 51299), two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), and one yeast strain (*Candida albicans* ATCC 90028). All reference strains were supplied by the Department of Medical Microbiology, Medical Faculty of Istanbul University. Sterile distilled water (50 µL) was used as a negative control, while 50 µL of ampicillin (25 µg/mL) was used as a positive control for bacterial pathogens and 50 µL of nystatin (200,000 units/mL) for fungal pathogens. The minimum inhibitory concentration (MIC) of the extracts was determined using the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI, 2018). Bacterial cultures were grown on Tryptic Soy Agar (TSA) (OXOID, Turkey), and fungal cultures were maintained on Sabouraud Dextrose Agar (OXOID, Turkey). All microbial strains were incubated aerobically at 35 °C for 24–48 hours. Bacterial suspensions were prepared in sterile saline (0.85% NaCl) and adjusted to a 0.5 McFarland standard (approximately 10^8 CFU/mL).

The antimicrobial assays were conducted in U-bottom 96-well microplates, with extracts tested at concentrations ranging from 5000 to 9 µg/mL and a final inoculum concentration of 1×10^5 CFU/mL. Mueller-Hinton Broth (MHB) (OXOID, Turkey) was used as the bacterial growth medium, while RPMI-1640 medium (Thermo Fisher Scientific, Turkey) was used for fungal cultures. To determine whether the extracts exhibit bactericidal or

bacteriostatic effects, future studies should include time-kill assays or viable cell count assessments after exposure to the extracts (Balouiri et al., 2016).

2.8. Cytotoxic activity

The cytotoxic potential of the extracts was assessed using three cell lines: NIH-3T3 (mouse embryonic fibroblast), K562 (chronic myeloid leukemia), and Saos (human osteosarcoma). All cell lines were obtained from the American Type Culture Collection (ATCC, USA). Doxorubicin (DOX; Sigma-Aldrich, USA, purity ≥98%) was used as a positive control to evaluate the cytotoxicity of the extracts, while untreated cells served as the negative control. NIH-3T3 cells were selected as a model for normal cell viability assessment (Rubin, 2001), ensuring that the extracts' cytotoxicity is specific to cancer cells. K562 cells were used to assess potential activity against hematological malignancies (Dutt and Madan, 2012), while Saos cells represented osteosarcoma as a solid tumor model (Czekanska et al., 2012). The IC50 values of the positive control were compared with those of the plant extracts (Injac et al., 2024). The IC_{50} values of the positive control and plant extracts were determined using the MTT assay ([3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide]) (Awang et al., 2023). Stock solutions of the extracts were prepared in DMSO (10 mg/mL) and further diluted with the cell culture medium to the desired concentrations. The cells were cultured in a 5% CO_2 incubator at 37°C for 72 hours. Following incubation, 10 µL of MTT solution (5 mg/mL) was added to each well, and the plates were incubated for an additional 4 hours. Formazan crystals were dissolved in isopropyl alcohol, and absorbance was measured at 570 nm using an ELISA reader. Each experiment was performed in triplicate. The IC_{50} (the concentration of extract required to inhibit 50% of cell growth) was calculated from the dose-response curve using nonlinear regression analysis. Lethal dose (LD_{50}) or lethal concentration (LC_{50}) criteria were not applied in this study, as these parameters require in vivo models or additional in vitro assays measuring long-term cytotoxicity and apoptotic effects.

3. Statistical Analysis

All experiments were performed in triplicate, and data were analyzed using ANOVA followed by Tukey's post hoc test ($p<0.05$) in GraphPad Prism 9 (GraphPad Software, 2020). One-way analysis of variance (ANOVA) was used to assess differences among groups, followed by Tukey's test for multiple comparisons ($p < 0.05$). Data are presented as mean ± standard deviation (SD) based on triplicate measurements.

4. Results and Discussion

The aerial parts exhibited higher flavonoid (5.88%) and organic acid (1.22%) contents, while the root extracts contained more saponins (0.87%). Ethanol extracts demonstrated the highest TPC, correlating with stronger antioxidant activity ($p<0.05$). The moisture content in the aerial parts was found to be 69.8%, whereas in the root parts, it was substantially lower at 10.1% (Table 1).

The total ash content, was higher in the aerial parts (19.1%) compared to the root parts (7.5%). Phytochemical studies reveal that flavonoids, tannins, saponins, and organic acids were very abundant in the aerial as well as root parts of *Z. fabago*. The aerial parts were particularly rich in flavonoids (5.88%) and organic acids (1.22%), which are known for their strong antioxidant and anti-inflammatory properties (Al-Khayri et al., 2022; Ferraz et al., 2020). The root parts had a slightly higher content of saponins (0.87%), which are known for their antimicrobial and immune-boosting effects (Feng et al., 2008; Iqbal et al., 2011). Previous studies have also identified new sulfated triterpenoids with antimicrobial properties (Feng et al., 2007) and disulfated triterpenoids, further contributing to the bioactive profile of the plant (Feng et al., 2010). *Tribulus terrestris* (*Zygophyllaceae*) is known for its rich phytochemical profile, which includes polyphenols, flavonoids, and saponins. Studies have reported that the concentration of polyphenols in *T.terrestris* ranges from 0.6% to 3%, while flavonoid content varies between 0.04% and 0.5% (Ștefănescu et al., 2020).

4.1. Total phenolic content

Maximum phenolic content was observed in ethanol extract of the aerial parts, which was 128.29 ± 0.81 mg GAE/g, followed by the ethyl acetate extract of the aerial parts, 115.54 ± 3.32 mg GAE/g. Minimum phenolic content, 19.72 ± 3.51 mg GAE/g (Table 2), was observed in the hexane extract of the roots. From the results, it's evident there's a vast difference in the type of solvent and parts of the plant on the phenolic content showing higher phenolic contents when polar solvents are used, ethanol, and ethyl acetate, compared to the non-polar hexane.

4.2. Antioxidant activities

The antioxidant activities of the extracts were evaluated using DPPH and ABTS assays, with Trolox employed as a standard reference for comparison of radical scavenging

Table 1. Phytochemical Composition of *Z. fabago* Parts.

Part	Moisture Content (%)	Total Ash Content (%)	Flavonoids (%)	Tannins (%)	Saponins (%)	Organic Acids (%)
Aerial parts	69.8	19.1	5.88	0.60	0.71	1.22
Root parts	10.1	7.5	4.00	0.51	0.87	0.52

efficiency. The DPPH radical scavenging activity of the ethanol extract from the roots (SC_{50} = 42.57 ± 3.03 µg/mL) was the highest among all extracts, indicating the presence of potent radical scavengers, likely due to its high phenolic content (128.29 ± 0.81 mg GAE/g). The ethanol extract from the aerial parts exhibited a SC_{50} value of 54.76 ± 5.72 µg/mL (Table 3), showing strong antioxidant activity. However, direct comparison with Trolox is not applicable, as Trolox is a pure synthetic compound, while plant extracts are complex mixtures of bioactive substances. The hexane extracts from both roots and aerial parts exhibited the weakest antioxidant activity, with SC_{50} values of 234.17 ± 13.06 µg/mL and 245.79 ± 5.94 µg/mL, respectively. In the ABTS assay, the ethanol extract of the roots showed the highest Trolox equivalent antioxidant capacity (TEAC), with a value of 261.63 ± 2.33 mg Trolox/g, followed by the ethyl acetate extract of the roots (247.78 ± 11.06 mg Trolox/g). The lowest activity was observed in the hexane extract of the aerial parts (72.68 ± 5.57 mg Trolox/g). Overall, this pattern indicates that ethanol and ethyl acetate solvent extracts, which contain more phenolic compounds, have more powerful antioxidant action than the hexane extracts. These findings are consistent with the observations of Feng et al. (2008), who stated that it was the triterpenoidal saponins responsible for the very high antioxidant activity of *Z. fabago*.

Table 2. Total phenolic content of *Z. fabago* extracts.

No.	Extract	Total phenolic content (mg GAE/g)
1	Roots Ethanol	111.34 ± 7.23a
2	Roots Ethylacetate	90.04 ± 3.80b
3	Roots Hexane	19.72 ± 3.51d
4	Aerial Parts Ethanol	128.29 ± 0.81a
5	Aerial Parts Ethylacetate	115.54 ± 3.32a
6	Aerial Parts Hexane	52.43 ± 2.59c

Values with different superscript letters (a, b, c, d) indicate significant differences between groups (ANOVA followed by Tukey's test, $p < 0.05$).

Table 3. Antioxidant activity test results of *Z. fabago* extracts.

Extract	DPPH SC_{50} (µg/mL) (Expressed as SC_{50} value)	ABTS (mg Trolox/g)
Roots Ethanol	42.57 ± 3.03c	261.63 ± 2.33a
Roots Ethylacetate	104.99 ± 4.83b	247.78 ± 11.06a
Roots Hexane	234.17 ± 13.06a	72.24 ± 2.80c
Aerial Parts Ethanol	54.76 ± 5.72c	186.21 ± 2.97b
Aerial Parts Ethylacetate	74.64 ± 4.70bc	153.07 ± 1.68b
Aerial Parts Hexane	245.79 ± 5.94a	72.68 ± 5.57c
Rutin	20.86 ± 3.47d	-

Values with different superscript letters (a, b, c, d) indicate significant differences between groups (ANOVA followed by Tukey's test, $p < 0.05$).

4.3. Antimicrobial activity of ethanol, ethyl acetate, and hexane extracts of Z. fabago

The extracts were tested for antimicrobial activity against five microbial strains, including two Gram-positive bacteria (*Staphylococcus aureus* ATCC 25923 and *Enterococcus faecalis* ATCC 51299), two Gram-negative bacteria (*Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853), and one yeast strain (*Candida albicans* ATCC 90028). Ampicillin (25 µg/mL) and nystatin (200,000 units/mL) were used as positive controls for bacterial and fungal pathogens, respectively. Among the tested extracts, the ethyl acetate extract of the aerial parts demonstrated the strongest antimicrobial activity, with MIC values of 39 µg/mL against *P.aeruginosa* and <9 µg/mL against *E.coli* and *C.albicans*. Ethanol extracts from both roots and aerial parts also showed notable antifungal activity, with MIC values of <9 µg/mL for *C.albicans* (Table 4). The antimicrobial effects observed in Z. *fabago* extracts may be attributed to the presence of bioactive triterpenoid saponins, which have previously been reported to exhibit antimicrobial properties (Feng et al., 2007). Notably, sulfated triterpenoidal saponins isolated from Z. *fabago* have demonstrated antimicrobial potential (Iqbal et al., 2011), suggesting that similar compounds may contribute to the activity observed in this study. Tribulus terrestris (Zygophyllaceae) has demonstrated notable antibacterial properties against both S.aureus and E.coli, the methanolic extracts of T.terrestris exhibit minimum inhibitory concentration (MIC) values ranging from 15 mg/mL to 20 mg/mL against E.coli and from 15 mg/mL to 25 mg/mL against S.aureus (Sharma et al., 2013). Additionally, ethanolic extracts from the fruits have shown potent activity, with MIC values as low as 0.62 mg/mL against E.coli (Al-bayati and Al-Mola, 2008). These findings suggest that Zygophyllaceae contains bioactive compounds effective in inhibiting the growth of pathogenic bacteria.

Two gram-negative bacteria that are often resistant to traditional antibiotics, P.aeruginosa (Lorusso et al., 2022) and E.coli (Medugu et al., 2022), were effectively inhibited by the ethyl acetate extract from the aerial parts. This indicates that the chemicals in the ethyl acetate extract have the ability to damage bacterial cell walls or interfere with vital bacterial functions. While the extracts showed promising antimicrobial activity, this study did not differentiate between bactericidal and bacteriostatic effects.

4.4. Cytotoxic activity and selectivity of ethanol, ethyl acetate, and hexane extracts of Z. fabago

The cytotoxicity of the extracts was evaluated using three cell lines: NIH-3T3, K562, and Saos, with DOX serving as the reference standard. The ethyl acetate extract from the roots exhibited the most potent cytotoxicity against K562 leukemia cells (IC50 = 121 µg/mL).

In contrast, the hexane solvent extracts from root and aerial parts showed weaker cytotoxic effects, with IC50 values exceeding 349 µg/mL. The ethanol extracts of the

Table 4. MIC values of *Z. fabago* extracts against microbial strains.

Microorganisms	Tested plant extracts (MIC μg/mL)							
Bacteria	**Ampicillin (Positive control)**	**Nystatin (Positive control)**	**Roots Ethanol**	**Roots Ethylacetate**	**Roots Hexane**	**Aerial Parts Ethanol**	**Aerial Parts Ethylacetate**	**Aerial Parts Hexane**
S. aureus ATCC 25923	2 μg/mL	-	2500	2500	2500	2500	2500	2500
E. faecalis ATCC 51299	2 μg/mL	-	2500	1250	625	2500	1250	1250
E. coli ATCC 25922	1 μg/mL	-	39	<9	78	156	78	156
P. aeruginosa ATT 27853	8 μg/mL	-	312	625	312	1250	39	1250
Fungi								
C. albicans ATCC 90028	-	4 μg/mL	<9	39	625	<9	312	312

roots and aerial parts displayed moderate cytotoxicity against K562 cells, with IC50 values of 168 μg/mL and 171 μg/mL (Table 5). These findings confirm the higher potency of the ethyl acetate extracts against cancer cells, especially the K562 cell line, and highlight their potential for further investigation as anticancer agents. The observed cytotoxic effects of ethyl acetate extracts on K562 and Saos cells suggest potential pro-apoptotic or anti-proliferative mechanisms, which require further molecular validation through apoptosis markers and gene expression analysis (Ziedan et al., 2008).

The results showed that the ethanol extract from the root parts exhibited the highest DPPH radical scavenging activity with an SC50 value of 42.57 ± 3.03 μg/mL. The ethyl acetate extracts also exhibited higher efficacy on the cancer cells, particularly the K562 cell line, and thus are recommended for further studies as anticancer agents. Since hexane is a non-polar solvent, this emphasizes that the nature of the bioactive polar or semi-polar compounds, such as phenolics, is better extracted with ethanol or ethyl acetate (Cravotto et al., 2022).

According to an Iranian study, significant antioxidant activity was observed in ethanol extracts of *Z. fabago*, particularly from the aerial parts, with an IC_{50} of 0.39 mg/mL in the DPPH assay (Yaripour et al., 2017). In our study, ethanol extracts demonstrated even stronger antioxidant activity. The highest activity was observed in the aerial parts (SC_{50} = 54.76 ± 5.72 μg/mL), which correlated with their high phenolic content. However, the root extracts also exhibited considerable phenolic content (128.29 ± 0.81 mg GAE/g), contributing to their antioxidant potential. Regarding cytotoxic activity, the two studies reported differing results. The Iranian study (Yaripour et al., 2017) concluded that *Z. fabago* extracts did not exhibit significant antiproliferative effects on cancer cells, suggesting that the aerial parts may not be promising for cancer research. In contrast, our study demonstrated moderate to strong cytotoxicity, particularly in the ethyl acetate extracts, which showed notable activity against K562 leukemia cells (IC_{50} = 120 μg/mL for aerial parts). This discrepancy is likely due to differences in cell lines and extraction methods used in the studies.

Table 5. Cytotoxic activity (IC50) of *Z. fabago* extracts.

Extract	IC50 (μg/mL)		
	NIH-3T3	**K562**	**Saos**
Doxorubicin (DOX)	100	20	15
Roots Ethanol	1800	168	161
Roots Ethylacetate	>2500	121	157
Roots Hexane	1800	349	420
Aerial Parts Ethanol	>2500	171	319
Aerial Parts Ethylacetate	1200	120	273
Aerial Parts Hexane	1800	379	416

In addition our ethyl acetate extracts demonstrated strong antimicrobial effects, particularly against *E.coli* and *C.albicans*, which were not explored in earlier studies (Feng et al., 2007, 2009). This difference in focus and methodology, including the use of both ABTS and DPPH assays in our study, underscores the potential of *Z. fabago* extracts in developing antimicrobial and anticancer agents.

5. Conclusion

This study highlights *Zygophyllum fabago* L. as a promising source of bioactive compounds with antioxidant, antimicrobial, and cytotoxic potential. Ethanol and ethyl acetate extracts demonstrated high total phenolic content, correlating with strong antioxidant activity. The ethyl acetate extracts, particularly from the aerial parts, exhibited potent antimicrobial effects, while their cytotoxicity

against K562 leukemia cells suggests potential anticancer applications. Future studies should focus on the isolation and structural characterization of active compounds, elucidation of their mechanisms of action, and in vivo validation of their therapeutic potential.

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