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Pharmacological attribute, LC-MS/MS profiling and *in-vitro* bioactivity of *Silene dichotoma* aqueous extract

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In this study, which aims to elucidate the nutritional value and biological activities of *Silene dichotoma*, an edible species, the anticancer, antioxidant, antimicrobial, and wound- healing activities of the plant were determined. Anticarcinogenic activity was investigated on A549 and SHSY-5Y cells, as well as in healthy HUVEC and L929 cell lines. Wound-healing activity was also investigated on L929 cells. Antimicrobial activity was evaluated against to *Staphylococcus aureus*, *Escherichia coli*, and *Candica albicans*. Mineral levels and phenolic content were determined. Based on the findings, it can be stated that extract applications at concentrations of 125 µg/mL-500 µg/mL would be effective anticarcinogenic doses in both A549 and SHSY-5Y cells. In L929 cells created as a wound model, the extracts showed an 82.51% wound closure effect. It was determined that the extract was at least as effective as the antibiotic against *E. coli*, but was not effective against *S. aureus.* On the fungal strain *C. albicans*, it was observed that high doses exhibited strong antifungal properties. The value of TAS activity was determined as 8.02 ± 1.47 mmol Trolox Equiv. /L, and the value of Total oxidant activity was determined as 17.9 ± 1.58 µmol H_2O_2 Equiv. /L. The minerals detected in the highest concentrations were Ca, Mg, and Fe, respectively. The most abundant phytochemicals were quinic acid, fumaric acid, and protocatechuic acid. In this study, which also included detailed content analysis, the species stands out for its anticarcinogenic and antibacterial activity against Gram-negative bacteria.

Keywords: Anticarcinogenic, Antimicrobial, Antioxidant, Bio-element, Phytochemical

Introduction

Cancer is a leading cause of death, leading to a high health burden. The International Agency for Research on Cancer reported a global prevalence of 29.8 million for 36 cancers in 2020, with 19.3 million new cancer cases and 10.0 million cancer-related deaths. The most frequently identified cancers were lung cancer, colorectal cancer, and breast cancer[1-3]. Lung cancer is currently the malignant tumor with the highest mortality rate worldwide. This is attributed to its often undetected nature and its inability to reduce patients' quality of life reduces at the onset significantly. Various factors, including active smoking, passive smoking, pipe and cigar smoking, exposure to indoor and outdoor air pollution, radiation exposure, and occupational exposure to heavy metals, have been implicated as possible causes of lung cancer. Non-small cell lung cancer, including lung cancer, squamous cell carcinoma, adenocarcinoma, and large cell carcinoma, account for 80% of all lung cancers. The remaining 20% is small-cell lung carcinoma[4-6].

Both traditional and modern techniques used to treat cancer. Various techniques, such as chemotherapy, radiation therapy, and surgery, are used in cancer treatment. However, all of these have certain disadvantages. The use of traditional chemicals leads to side effects and toxicities. Due to the drawbacks of traditional chemotherapeutic approaches, new approaches to disease control are needed. Therefore, new strategies for cancer prevention and treatment are necessary to control the mortality rate associated with this disease[7].

The treatment of chronic wounds is one of the most complex therapeutic problems in medicine. Burn wounds, chronic wounds, and diabetic foot ulcers are wounds that take a long time to heal despite appropriate treatment. These wounds are accompanied by chronic

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inflammation resulting from the suppression of both humoral and cellular immune response. This increases susceptibility to bacterial infections, further delaying and hindering proper wound healing. Commercially available therapeutic agents maintain wound moisture or prevent bacterial infection. However, they contain compounds that do not stimulate skin regeneration processes. Properly selected active compounds should influence the biochemical processes occurring in each of the four phases of wound healing: wound cleansing, inflammation, proliferative, and wound maturation. Active compounds that have multifaceted effects on the staes of wound healing and also possess antimicrobial properties may be beneficial in the treatment of chronic wounds[8-10].

Herbal medicines have become a highly safe, non-toxic, and readily available source of compounds used in the treatment of cancer and chronic wounds. Plants are believed to neutralize the effects of diseases in the body due to their various properties[11].

Silene dichotoma belongs to the Caryophyllaceae family and is a 10-30 cm tall plant species that grows wild. *S. dichotoma* is widespread in Turkey, Cyprus, Palestine, the Caucasus, Northern Iran, Africa, and most of Europe. This plant is frequently used in salads or as a dish by the people of Anatolia, where it grows naturally from April to June. Although *S. dichotoma* is an edible species, no studies have been conducted on it. Therefore, the present study was designed to elucidate both the nutritional value and biological activities of plant. The present study investigates the anticarcinogenic and wound-healing activities of *S. dichotoma* for the first time. Furthermore, the species' phenolic and mineral content were determined in detail for the first time. The data obtained as a result of this study may contribute to the explanation of the possible benefits in nutrition and treatment of diseases by revealing the components and biological properties of the *S. dichotoma* plant used as a food source.

Materials and methods

The chemical and biological properties of *Silene dichotoma* were investigated. The cytotoxic effects of an aqueous extract of *Silene dichotoma* were investigated on A549 (lung cancer cell), SHSY-5Y (human neuroblastoma cell), HUVEC (human umbilical vein endothelial cell), and L929 (fibroblast-like cell derived from mouse subcutaneous connective tissue) cell lines. The wound healing activity on L929 cells was also investigated. The plant's antimicrobial, antioxidant, and phytochemical constituents were also investigated.

Plant collection and extract preparation

The *Silene dichotoma* plant used in this study was obtained from the town of Şuhut in Afyonkarahisar province between April and May. The species identification was carried out by Prof. Dr. Mustafa Kargıoğlu. The obtained *S. dichotoma* plant was washed with distilled water, cleaned of soil, dried, and ground with a blender in a mix (branches and leaves) to form powder. It was stored in a glass jar in the dark at room temperature. 25 grams of the prepared homogenized powdered plant was weighed into a clean bottle and 250 mL (1:10 w/v ratio) of deionized water was added. It was then incubated in an ultrasonic water bath at 60 ºC for 2 hours and stirred on a magnetic stirrer at 700 rpm at 60 ºC for two hours. It was then kept in the dark for 24 hours at room temperature. The prepared aqueous extract was filtered with filter paper (Whatman, Grade 589/1) and a sieve flask and collected in a separate flask. The solvent of the aqueous plant extract was removed with an evaporator device under vacuum (D-LAB). The extract obtained in this manner was stored at -18°C until analysis.

Cell culture medium preparation, cultivation, and counting

The complete medium used for cell growth was prepared containing 1% glutamine, 10% fetal bovine serum (FBS), and 1% penicillin and streptomycin. DMEM was used for A549, SHSY-5Y, and L929 cell cultures, while RPMI 1640 was used for HUVEC cells. Cells were added to a 500 µL flask. The cells were then transferred to an incubator (37°C and 5% CO2, Nuve EC 160). Cell flasks were regularly observed under an inverted microscope (VWR ECN: 630-2738), and the cell culture medium was changed every two days until the cells reached 80-90% confluence. Cell counts were required before inoculation. The number of cells per mL of the resulting cell suspension was determined by counting cells. In the presented study, the trypan blue method was used for cell counting. Trypan blue is a staining method that allows viable cells to appear bright under light. The cells to be counted were placed on a Thoma slide, and when examined under a microscope with trypan blue stain, viable cells appeared bright, while non-viable cells had a dull bluish color. Using the dilution factor and the average cell count, the cell count per mL was determined using the formula presented below[12].

Viable cell count per mL = (average cells) x (10^4) x dilution factor

Cell viability assays

The MTT assay determines the cytotoxicity of plant extracts applied to living cells[13]. After sufficient growth of the cells (A549, HUVEC, SHSY-5Y, and L929), they were seeded in 96-well plates with 10^4 cells per well. Twenty-four hours later, plant extract solutions prepared at seven different concentrations (15.625, 31.25, 62.5, 125, 250, 500, and 1000 µg/mL) were added to the predetermined wells in three replicates. Incubation was left for one day. At the end of incubation, MTT solution (20 µL) at a concentration of 5 mg/mL in phosphate buffer (pH= 7.4) was added to each well. At the end of incubation, the medium in the wells was aspirated without damaging the crystals formed at the bottom of the well. 200 µL of dimethyl sulfoxide (DMSO) was added to the wells. Finally, the absorbance of each well was measured at 540 nm using a multiplate reader (Biotek, ELx800). Cell viability in the control group (which received no substance) and the experimental groups was calculated using the formula given below[12].

Cell Viability (%) = [(100 * Absorbance $_{sample}$) / (Absorbance $_{control}$)]

Determination of wound healing activity

L929 mouse fibroblast cells, a type of fibroblast cell, were used in the analysis of wound healing activity in the presented study. The effect of the materials used in the study on wound healing was assessed using the in vitro cell scratch wound healing test[14]. For this purpose, sufficiently proliferated cells were seeded into tissue culture well plates at a concentration of $2x10^4$ cells/mL in growth medium.

The cells were cultured until they reached confluence at the bottom of the culture dish. A wound model was then created by making a linear scratch on the cell layer at the bottom of each well using a 200 µL sterile plastic pipette tip. After removing the medium from the wells, 1000 µL of media containing 10 µg/mL of active ingredient from each extract was added to the wells. The same volume of medium and extract solvents was added to the control wells. The cells were then placed in the incubator. Images of each well were recorded at the beginning (zero hour) and at 24 hours. Studies were performed in at least three replicates. The images obtained at the end of the incubation were contoured using Image J software (determining the boundary of the cell-free area), and then the percentage of cell-free area (wound) between them was calculated based on pixels. The potential effect of each material applied to the cells in the study on wound healing was determined by comparing the percentage of cell-free area in the control group with the percentage of cell-free area in the other groups[14].

Determination of antimicrobial activity

The antimicrobial activity of the mixed aqueous extract of *S. dichotoma* was determined using the disk diffusion method[15]. A gram-positive bacterium, *Staphylococcus aureus* (ATCC 25923), a gram-negative bacterium, *Escherichia coli* (ATCC 43888), and a fungal strain, *Candida albicans* (ATCC 90028), were used as reference strains. The reference strains were diluted using the dilution method, and inocula were prepared at concentrations consistent with the 0.5 McFarland turbidity standards (10^6-10^7 cfu/mL). The prepared inocula were added in 750 µL volumes to sterile Petri dishes previously prepared with Mueller-Hilton agar (Merck), ensuring even distribution on the agar. After culturing the microorganism strains in this manner, the inoculum was left in the dark for 2-4 hours to ensure that the agar thoroughly absorbed microorganism-containing inoculum. Then, sterile, empty discs with a diameter of 6 mm were placed in petri dishes. 10 µL of *S. dichotoma* extract solutions prepared in deionized water at various concentrations (ranging from 10 to 1000 µg/mL) were applied to the discs. Discs containing 10 µg of penicillin were used as the standard. After the applications, the petri dishes were placed in an incubator at 37°C. Studies were conducted in at least three replicates. After incubation for one day, the petri dishes were removed from the incubator, and the inhibition zones (mm) surrounding the discs were measured.

Determination of mineral content levels

To determine the mineral content of *Silene dichotoma* plants, the organic components must first be removed from the plant. For this purpose, wet digestion method was used to decompose organic compounds and convert inorganic compounds into soluble forms. The wet digestion process was carried out with the help of appropriate acids using a microwave system (Berghof, Speedwave) in our department laboratories. 0.1 gram of *S. dichotoma* plant was weighed and placed in Teflon wells belonging to the microwave oven. 3 mL of nitric acid, 1 mL of hydrogen peroxide, and 0.5 mL of perchloric acid were added. The lids of the Teflon wells were

closed and placed in the microwave oven. The digestion process was carried out in the microwave oven at 75 °C/5 min., 160 °C/15 min., and 75 °C/5 min. The samples in the wells were transferred to a volumetric flask (10 mL). Ultrapure water (18.2 MΩxcm) was added to make the final volume up to 10 mL. Mineral substance (micro and macro element) concentrations in the samples were measured using the ICP-OES (Spectro Genesis, Germany) system. Multi-element standards (Merck, ICP multi-element standard solution XVI; Merck, ICP multi-element standard solution IV) were used for method calibration. Calibration curves were generated for the elements in the standards and the levels of the elements detected in the samples were expressed in mg/L (ppm) [16].

Determination of total phenolic content and phenolic components of *Silene dichotoma*

The total phenolic content of extracts obtained from *S. dichotoma* was determined using the Folin-Ciocalteu method[17]. Gallic acid solutions at different concentrations were chosen as standards. Folin-Ciocalteu reagent was added to the standard extract solutions first, followed by Na_2CO_3. The absorbance of the samples, which were incubated in the dark for 30 minutes, was measured at 760 nm using a spectrophotometer (Shimadzu 1700).

The phenolic compounds of the *S.dichotoma* aqueous extract were determined using an LC-MS/MS system. For this purpose, a validated method using 53 standards was chosen. [18, 19] internal standard solutions were used to increase the reliability of the results by compensating for matrix effects and analyte losses during sample preparation and analysis. Rutin D3, quercetin D3, and ferulic acid D3, deuterated (flavonoid glycosides, flavonoids, and non-flavonoids, respectively), were used as internal standards. Method validation procedures were described in terms of repeatability (within-day and between-day precision), linearity, recovery (accuracy), detection and LOD/LOQ (limits of quantification), and relative standard uncertainty (U% (k=2) at the 95% confidence level) [18]. Plant extracts weighing in the 5-10 mg range and prepared in deionized water were run through the LC-MS/MS system. The chromatograms obtained were compared with the chromatograms of the standard substances to determine whether each standard substance was present in the plant extract and, if so, at what concentration. The components determined in the aqueous extract of *S. dichotoma* were expressed as mg-analyte/g-extract[19].

Determination of oxidative/antioxidant capacity of *Silene dichotoma* extract

To determine the antioxidant and oxidant activities of *Silene dichotoma* extract, solutions were prepared at a concentration of 1 mg/mL in their respective solvents, and the solvents served as blanks in the analyses. To determine oxidant/antioxidant activity, TAS and TOS were measured using commercially available kits (Rell Assay, Gaziantep, Turkey) available in our laboratory. Trolox was used as a standard for determining TAS levels, and vitamin C was used as a reference. The TAS levels of the determined *S. dichotoma* plant extracts were compared with the antioxidant activity of vitamin C. A calibration curve drawn using H2O2 standards was used to determine TOS levels. The oxidative stress index (OSI) was calculated from the TAS and TOS data by dividing the TAS levels by the TOS levels[20, 21].

Statistical Analyses

SPSS 20, a statistical program, was used to calculate and evaluate the data obtained from our studies on the *S. dichotoma* plant. After testing the data for normal distribution, we applied the parametric one-way analysis of variance (ANOVA) and the Duncan test as a post hoc test to the normally distributed data. Data showing statistical differences and identifying differences between groups are marked with an (*) symbol.

Result and discussions

An aqueous extract of *S. dichotoma* stems and leaves was prepared and its anticarcinogenic effects (on cancer cell lines A549 and SHSY-5Y, and healthy cell lines HUVEC and L929), antimicrobial effects, wound healing properties, and mineral and phenolic compound contents were investigated.

Anticarcinogenic activity

Understanding cancer mechanisms plays a crucial role in developing diagnostic and therapeutic strategies, and as well as preventing cancer. Studying the characteristics exhibited by cancer cells in vitro provides valuable insights into the molecular processes that lead to tumor formation. Cell culture techniques are crucial in cancer research. These techniques allow for the manipulation and observation of cancer cells in a controlled environment[22, 23]. The

study investigated the cytotoxic effects on A549 (lung cancer cell line), SHSY-5Y (a human neuroblastoma cell line), HUVEC (human umbilical vein endothelial cells), and L929 (a fibroblast-like cell line derived from mouse subcutaneous connective tissue).

Four cell lines were studied for anticarcinogenic activity. These were A549, SHSY-5Y, HUVEC, and L929. Cytotoxicity refers to the inhibition of cellular activity in in vitro studies. Seven different application doses were used to evaluate the anticarcinogenic activity of the cells and their effects on cell viability. The concentrations used ranged from 15.625 to 1000 µg/mL. Figure 1 shows the cytotoxic and anticarcinogenic effects of *S. dichotoma* aqueous extract on A549, SHSY-5Y, HUVEC, and L929 cells.

When examined in A549 cells, the extract applied at all concentrations except the lowest (15.625 µg/mL) reduced cell viability in cancer cells. This strain can be said to have significant anticarcinogenic effects on lung cancer cells. In SHSY-5Y cells, the extract was found to increase cell proliferation at low concentrations, had no effect at 62.5 µg/mL, but was an effective anticarcinogen at all other high concentrations (125-1000 µg/mL).

A 1000 µg/mL concentration of the extract applied to healthy HUVEC cells reduced cell viability, while other doses were ineffective. The extract increased cell viability at lower doses applied to L929 cells, while higher doses were ineffective.

In this case, it can be said that extract application at concentrations of 125 µg/mL, 250 µg/mL, and 500 µg/mL would be effective anticarcinogenic doses on both A549 and SHSY-5Y cells. Although the 1000 µg/mL application also has anticarcinogenic effects, it also appears to reduce the viability of healthy HUVEC cells.

In a study investigating the anticancer activity of *Silene viscidula*, a traditional Chinese herb from the same family, 16 compounds were obtained from the species. Among these compounds, cynocrassulosides VI/VII, an isomer mixture, exhibited the most potent inhibitory activity aganist HeLa cells with an IC50 value of 2.37 µM. Mechanistic studies have demonstrated that cynocrassulosides VI/VII induce cell cycle arrest in HeLa cells by decreasing cyclin D1 expression and retinoblastoma (Rb) phosphorylation[24]. *Silene dichotoma* has exhibited anticancer effects, consistent with the literature, similar to *Silene viscidula*, which belongs to the same family.

Wound healing activity

Wound healing is a complex process that involves the coordinated actions of numerous tissues and cells. It requires the strict regulation of cell migration, proliferation, matrix deposition, and remodeling, as well as inflammation and angiogenesis. While minor skin wounds heal within days, larger wounds resulting from trauma, acute illness, or major surgery can take several weeks to heal and often leave a fibrotic scar that can affect tissue function. Wounds heal by repair and/or regeneration. Repair is the physiological adaptation of damaged tissue after injury to restore continuity, regardless of whether the damaged tissue is completely replaced. Regeneration refers to the replacement of damaged tissue with replica, thus fully restoring both morphology and functionality. Mammalian skin does not regenerate spontaneously; it heals through scars (repair) [25, 26]. Fibroblasts, a type of connective tissue cell, play a crucial role in wound healing. Therefore, fibroblast cells are frequently used to investigate the wound-healing efficacy of a substance in vitro. In the presented study, the wound-healing effects of mixed aqueous extracts of the *S. dichotoma* were investigated using L929 cells, a fibroblast cell line. The analysis results are presented in Figure 2 and Table 1.

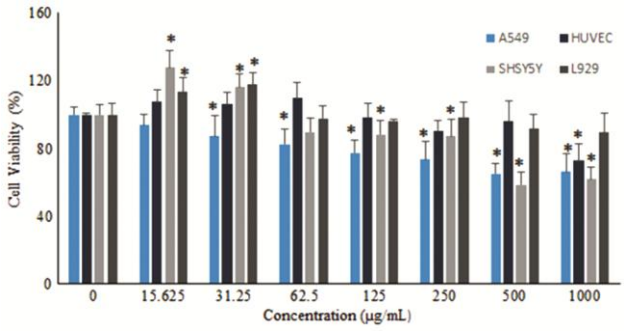

Fig. 1 — Cytotoxic and anticarcinogenic effects of *Silene dichotoma* extract on A549, SHSY-5Y, HUVEC, and L929 cells

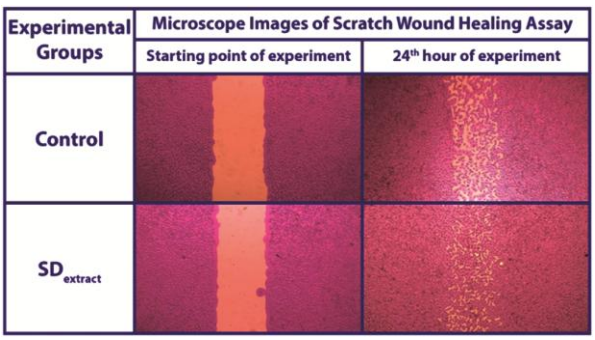

Fig. 2 — Effect of *Silene dichotoma* aqueous extract on wound healing in L929 cells

Table 1 —Wound-healing efficacy of the *Silene dichotoma* aqueous extract

	Amount of cell-free area (wound) (%)		Closing Rate (%)
	0. Hour	24. Hour	
Control	18.34±3.25	7.57±1.90	59.06±3.19
$SD_{extract}$	19.62±2.49	3.35±0.56*	82.51±5.12*
P	0.841	0.023	0.004

Data are presented as mean ± standard deviation (n≥3). * indicates data with statistically significant ($p<0.05$) better wound healing efficacy than the control group.

The close acellular areas (between 18.34% and 19.62%) in the wound models created for each experimental group at the beginning of the study demonstrate that the in vitro cell scratch wound model was successfully created. When the wound closure rates of the control group and the cells treated with the *S. dichotoma* mix aqueous extract were examined after 24 hours, it was observed that the *S. dichotoma* extract had an 82.51% wound closure effect, and its closure was statistically significantly higher than that ofthe control group ($p<0.05$).

The preventive activity of 70% ethanol extracts of *Silene viridiflora* and *Silene frivaldszkyana* against ulcer wound was investigated. *S. viridiflora* and *S. frivaldszkyana* extracts were administered to mice at doses of 50, 100, and 200 mg/kg, and to rats at doses of 25 and 50 mg/kg. To evaluate ulceration-preventive activity, the percentage of ulcers in the affected animals was determined, and the ulceration-preventive activity was compared with the control group. In the study on rats, the total effect of S. viridiflora and the effects of the extracts administered at doses of 25 and 50 mg/kg were calculated as 0.9, 1.7, and 1.1, respectively. The ulceration-preventive activity of famotidine, used as a positive control, was calculated as 2.3 in rats. Based on these values, it was concluded that *S. viridiflora* has preventive activity against ulcer woun and exhibits gastric protective effects[27] .Like *S. viridiflora*, *S. dichotoma* also has positive effects on wound healing.

Antimicrobial activity

Preventing pathogens that cause food spoilage and poisoning is generally achieved through the use of chemical preservatives. In addition to the harm these preservatives pose to human health, they also have adverse effects includin the introduction of chemical residues into the food and feed chains and the development of microbial resistance to the chemicals used. Therefore, the need to find potentially effective, healthy, safe, and natural alternative preservatives is increasing. Plant extracts are being used to control food poisoning illnesses and preserve food[28].

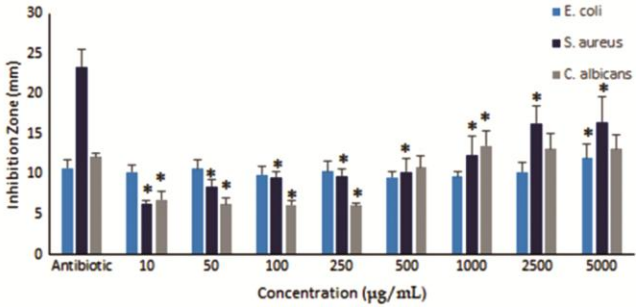

Fig. 3 — Antimicrobial activity of *Silene dichotoma*

The antimicrobial activity of *S. dichotoma* aqueous extract was determined using strains of *Escherichia coli*, *Staphylococcus aureus*, and *Candida albicans*. Eight different concentrations (10-5000 µg/mL) of *S. dichotoma* aqueous extract were used for this purpose. Disks containing antibiotics (penicillin, 5 IU) were used as positive controls. The results are presented in Figure 3.

When the effect of *S. dichotoma* extract on Gram-negative bacteria was examined, it was found to be as effective as the antibiotic at all concentrations except 5000 µg/mL, and was even more effective than the antibiotic at this concentrations. It was determined that the extract was less effective than the antibiotic at any dose against *S. aureus*. On the fungal strain C. *albicans*, low doses were ineffective, while higher doses exhibited strong antifungal properties. Even at a concentration of 1000 µg/mL, it exhibited greater antifungal activity than the antibiotic. A general evaluation of the antimicrobial activity of *S. dichotoma* indicates that it exhibits antibacterial properties against Gram-negative bacteria and strong antifungal activity against fungi.

In a study conducted with *Silene multifida*, samples were extracted with chloroform ($CHCl_3$). The chloroform extract was dissolved in dimethyl sulfoxide (DMSO), and six fractions were obtained after column chromatography. These fractions were prepared at different concentrations and tested for antimicrobial activity against six bacteria (*B. subtilis*, *S. aureus*, *E. coli*, *P. aeruginosa*, *E. cloacae*, and

P. vulgaris) and one fungus (*C. albicans*) using the agar diffusion technique. The *S. multifida* extract demonstrated activity against all bacteria tested at all concentrations. Antifungal activity has been determined to vary depending on concentration[29]. The presented study is effective against *E. coli* and *C. albicans*, in parallel with the literature, and it can be said that *S. dichotoma* has an antimicrobial effect similar to *Silene multifida*.

Mineral substances and their concentrations in *Silene dichotoma*

It is a common belief that some plants are rich in specific nutrients, particularly mineral nutrients. Any study on human nutrition often cites certain sources as rich sources of a mineral. Dark leafy greens such as broccoli, green beans, kale, and spinach are cited as "iron-rich foods." Similarly, legumes, nuts, and whole grains are cited as sources of zinc, a vital micronutrient for both plant and human health[30].

Mineral analysis of *S. dichotoma* was performed using the ICP-OES system. 14 minerals were detected in the sample. Mineral substances and their concentrations in *Silene dichotoma* were shown Table 2. The highest concentration was Ca, followed by Mg and Fe. Additionally, the concentrations of Mn and Zn, which are essential microelements involved in antioxidant enzymes, are also observed to be quite high.

In a study in the literature, the metal tolerance of facultative metallophytes *Biscutella laevigata* and *Silene vulgaris* was evaluated using bioconcentration factor and the translocation factor. In *S. vulgaris*, Cd: 0.01-1.60 mg kg^{-1}, Pb: 0.01-138 mg kg^{-1}, Tl: 0.01-578 mg kg^{-1} and Zn; 14.9-1590 mg kg^{-1} were detected. In terms of phytoremediation potential, both species were confirmed to be promising; however, *S. vulgaris* should be preferred over *B. laevigata* for phytostabilization projects in substrates with low Tl concentrations. The high concentrations of PTE found in the sediments of Rio del Lago/Slizza and in herbaceous plants collected from the banks of the waterway raise concerns about the potential impact of these elements on the surrounding ecosystem[31].

Metal content in the leaves and stems of *Silene alba* was analyzed. The content of some metals (Ca, Zn, Mn, Fe, Ni, Pb, Cr) was determined using atomic absorption spectrometry. Heavy metal content was found to be within acceptable normal limits, including Pb and Cr markers for soil and air pollution. Higher levels of Ca, Zn, Mn, Fe, and Cr were detected in the stems compared to the leaves. The results indicate that *Silene alba* can be used in pharmaceutical preparations[32]. In the presented study, Ca and Fe were found in high concentrations. Other identified metals (Mn, Zn, Ni, and Cr) are also present in *Silene dichotoma.* However, the element Pb has not been found in *S. dichotoma.*

Phenolic compounds detected in the aqueous extract of *Silene dichotoma*

Phenols are widely distributed in plants and are the most abundant secondary metabolites of plants. Plant polyphenols are attracting increasing attention due to their potent antioxidant properties and their significant effects in preventing various diseases related to oxidative stress such as cancer. In recent years, the identification and development of phenolic compounds or extracts from various plants has become an important area of health and medical research. Phenolic extraction and quantification provide comprehensive information about their antioxidant properties[33].

The total phenolic content of extracts obtained from *S. dichotoma* was determined using the Folin-Ciocalteu method. Gallic acid solutions of different concentrations were used, and the total phenolic content was determined to be 123.28±6.57 mg GAE/g extract.

The phenolic compounds of *S. dichotoma* were investigated using LC-MS/MS with 53 standards, and the presence of 7 of these compounds was detected. The highest concentrations were for Quinic acid at 7.256 µg analyte/mg extract, Fumaric acid at 0.419 µg

Table 2 — Mineral substances and their concentrations in *Silene dichotoma*

Element	Concentration (mg/L)	Element	Concentration (mg/L)
Be	0.11	Mn	38.90
Ca	12967.2	Mo	1.16
Cr	3.42	Ni	1.91
Co	0.28	Sr	98.04
Cu	18.19	Ti	62.06
Fe	1204.46	V	3.67
Mg	2757.64	Zn	57.06

analyte/mg extract, and protocatechuic acid at 0.061 µg analyte/mg extract. The amounts of all detected compounds are presented in Table 3.

In the total phenolic content analysis conducted for the *Silene dichotoma* plant, the highest-containing compound was identified as quinic acid. Quinic acid is an important phenolic compound. Phenolic compounds are classified as important antioxidants. Antioxidants, on the other hand, act on cells in diseases such as cancer, binding free radicals. Thus, they prevent the damage cause by these radicals which would otherwise negatively affect cells, thereby maintaining cell viability. Phenolic substances, obtained from plants, support the body's defense mechanism. After quinic acid, the highest-containing compound in *S. dichotoma* and one of the most important phenolic substances is fumaric acid. Fumaric acid is an organic compound with a fruity flavor. *S. dichotoma* is also rich in protocatechuic acid. It has a significant antioxidant effect on cancer cells and is known to positively affect cell proliferation (cell division and growth) and inhibit apoptosis (physiological cell death) of stem cells.

A comprehensive metabolite profile of the medicinal plant *Silene viridiflora* using UHPLC-ESI-MS/MS is described for the first time. A total of 71 compounds were identified and described; the most common of these are flavonoids, triterpene glycosides, and ecdysteroids. Three main compounds, shaftoside, 26-hydroxyecdysone, and silviridose, are reported to be suitable markers for assessing the quality of *S. viridiflora* preparations[34]. The studied species contained nonphenolic acids such as quinic acid, fumaric acid, and aconitic acid, phenolic acids such as gallic acid, protocatechuic acid, and salicylic acid, as well as phytochemicals belonging to the coumarin (benzoprone group), but no flavonoids were found.

A study conducted with *Silene ruscifolia* revealed that the species contains rutin, narcissin, luteolin, isorhamnetin, rhamnetin, and quercetin dimethyl ether. GC-MS analysis revealed that the extract had the highest content of sugars (50.5%) and sugar alcohols (46.39%). It also contains carboxylic acid (0.47%), fatty acid (0.64%), sugar acid (0.42%), glycoside (0.48%), carotenoids (0.61%), and benzoic acid ester (0.49%). D-pinitol has the highest content in the extract at 41.14%[35].

Table 3 — Components Detected in *Silene dichotoma* Extract

Components	Concentration (µg analyte/mg extract)
Quinic acid	7.256
Fumaric acid	0.419
Aconitic acid	0.024
Gallik acid	0.025
Protocatechuic acid	0.061
Coumarin	0.013
Salicylic acid	0.027

TAS, TOS, and OSI values of *Silene dichotoma*

Phenolic compounds, particularly due to their antioxidant activity, have positive effects associated with fruit and vegetable consumption. One study investigated the antioxidant activity, formation, and utilization of phenolic compounds in plants and agro-industrial byproducts. Fruits, vegetables, and beverages are the primary sources of phenolic compounds in the human diet. Plant polyphenols, as dietary antioxidants, can protect against oxidative damage in human health and disease. As natural antioxidants, phenolic compounds are abundant in plant foods, which play a vital role in nutrition and healthcare. Phenolic compounds are attracting attention due to their role in preventing various human diseases[36-41]. Antioxidants are compounds that help organisms eliminate oxidative stress caused by free radicals. Antioxidants are substances that contain phenolic functions. Antioxidants form a defense mechanism against toxic chemical substances in the body. The body requires antioxidants to reduce the risk of many diseases, especially cancer. The protective effects of antioxidants produced by the body are more limited. Antioxidants taken from foods are more effective. Compounds such as vitamins C, E, and beta-carotene, found in fruits, in particular, contain high amounts of antioxidant properties. While some antioxidants can be obtained from plants through dietary supplements, others are produced by the body as a defense system against free radicals. Therefore, antioxidants play a crucial role in human health[14].

The total antioxidant level (TAS) of the *Silene dichotoma* plant is a parameter that measures the body's total antioxidant capacity against free radicals. The TAS parameter was measured using commercially available kits. The TAS activity value was found to be 8.02 ± 1.47 mmol Trolox Equiv./L. Total oxidant level (TOS) is a measure of the oxidant capacity of the body. The TOS parameter was also measured using commercially available kits. The total oxidant activity value was found to be 17.9 ± 1.58

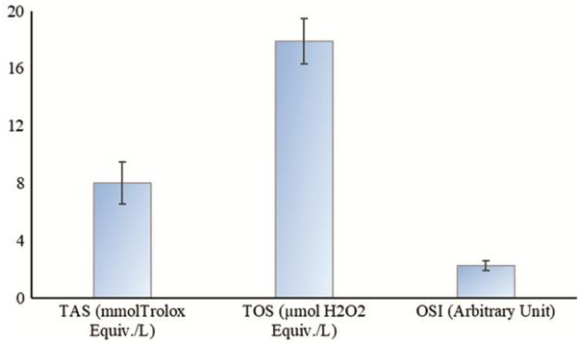

Fig. 4 — TAS, TOS and OSI values of *Silene dichotoma*

µmol H_2O_2 Equiv/L. Oxidative stress index (OSI) is an indicator of oxidative stress exhibited by the body. It is the parameter measured by the ratio of TAS and TOS levels to each other. The numerical value of the oxidative stress index was found to be 2.27 ± 0.33 arbitrary units (Figure 4).

A previous study in the literature used *Silene compata*, a member of the same genus. Four different *S. compata* extracts (petroleum ether, acetone, methanol, and water) were prepared, and the ABTS radical scavenging activity of the extracts, along with their total phenolic and flavonoid contents, were determined. The ABTS radical scavenging activity of the aqueous *S. compata* extract was found to be 55.45 ± 2.18 µg/mL. Total phenolic and flavonoid contents were expressed as pyrocatechol (PEs) and quercetin (QEs) equivalents. The methanol (SCM) extract showed the highest phenolic content (108.94 ± 2.68), while the aqueous extract had the lowest phenolic content (72.56 ± 0.06). This study in the literature indicates that the methanolic extract exhibited better antioxidant activity[42].

The phenolic, fatty acid, and essential oil profiles of *Silene compacta* Fischer (SC), as well as its cytotoxic, antioxidant, and cholinesterase inhibitory activities and total phenolic-flavonoid content, were investigated. In this study, the essential oil and fatty acid compositions of S. compacta were determined using GC/MS. The chemical composition of the methanol extract was determined using LC-MS/MS for quantitative and qualitative purposes. The main components of the essential oil and fatty acid were identified as α-selinene (12.4%) and palmitic acid (26.3%), respectively. Among the extracts tested, the methanol extract (SCM: *S. compacta* methanol extract), which had the best activity in four antioxidant methods, exhibited very strong cholinesterase inhibitory activities. Furthermore, this extract exhibited the highest cytotoxic effect against A549 cells. In the SCM extract, hesperidin and rutin, quinic, and malic acids were identified as the main components by LC-MS/MS[42]. Quinic acid was identified in *Silene dichotoma*, also.

The study examined the chemical composition, antioxidant, and enzyme inhibitory activities of water, methanol, and ethyl acetate extracts obtained from *Silene compacta*. The methanol extract showed the highest total phenolic (30.88 mg GAE/g) and flavonoid (50.19 mg RE/g) content, demonstrating superior antioxidant activities in DPPH• (45.82 mg TE/g) and ABTS•+ (57.03 mg TE/g) assays, as well as in CUPRAC and FRAP assays. In contrast, despite its low phenolic content, the ethyl acetate extract exhibited remarkable activity in the phosphomolybdenum assay (275.10 mg TE/g) and exhibited particularly strong enzyme inhibitory activities against α-glucosidase (1470.25 mg ACE/g) and AChE (3.11 mg GALAE/g). A water extract with a moderate phenolic content exhibited balanced antioxidant properties in various experiments, but its enzyme inhibitory effects were weaker. Correlation analysis revealed strong positive relationships between total phenolic content and most antioxidant assays, highlighting the importance of phenolic compounds contributing to the observed bioactivities. The findings suggest that methanol extracts are particularly promising for applications requiring strong antioxidant properties, while ethyl acetate extracts may be more suitable for applications involving enzyme inhibition. Future research should consider in vivo studies and investigate the synergistic effects between different phytochemicals to fully understand the therapeutic potential of *S. compacta*[43].

Conclusion

Silene dichotoma, whose phytochemical components and some pharmacological properties were investigated in this study, is rich in minerals such as Fe, Mg, Zn, and Mn, indicating its high nutritional value. It can be argued that these elements, which are said to be present in the antioxidant structure, may support both metabolism and the immune system. The phenolic compounds contained in the extract positively affect the antioxidant capacity of the species. It is known that plants with high phenolic compounds may have high antioxidant activity, and these properties may contribute to antimicrobial and anticarcinogenic activity. The strong anticarcinogenic and antimicrobial activity of

Silene dichotoma aqueous extracts may be related to its oxidant/antioxidant activity. In conclusion, this study, which examined the chemical composition and biological activities of *Silene dichotoma*, determined that the species contains high concentrations of Ca, Mg, and Fe, and that the most abundant phytochemicals in its structure are quinic acid, fumaric acid, and protocatechuic acid. It can be said that the application of certain extract doses would be effective anticarcinogenic doses on both A549 and SHSY-5Y cells. It was observed to exhibit effective wound-sealing activity. The extract was found to be at least as effective as antibiotics against *E. coli* but not against *S. aureus*. Higher doses were observed to exhibit strong antifungal properties against the fungal strain *C. albicans*. The anticarcinogenic, antimicrobial, antioxidative, and wound-healing properties of *Silene dichotoma* have been identified and reported in the literature for the first time. Furthermore, content studies have further contributed to the plant's potential nutritional value.

Conflicts of interest

The authors declare no conflicts of interest.

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