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# Investigation of the bioactivities of *Saponaria mesogitana* methanolic extract along with its phytochemical composition

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*Abstract*: *Saponaria* species are known to contain saponins which have a wide variety of biological activities. But up to now, the phenolic compounds of *Saponaria mesogitana* have not been clarified. Therefore, this study aimed to determine the phenolic composition and some biological activities of *S. mesogitana* for the first time. The antioxidant activities of the methanol and water extracts were assessed using the DPPH, FRAP and *β*-carotene/linoleic acid assays, while the total secondary metabolite content, including phenolics, flavonoids and saponins, was also determined for both extracts. Based on the antioxidant activity and total phenolic and flavonoid contents, further HPLC analysis, as well as anticancer and antimicrobial activity experiments, were conducted using the methanol extract. The anticancer potential was assessed using the MTT assay and wound healing migration test, while antibacterial activity was evaluated through disc diffusion and MIC assays. Additionally, the antibiofilm properties of the extract were examined using the crystal violet method. The methanolic extract showed high antioxidant activity, while caffeic acid and epicatechin were characterized as major phenolic compounds by HPLC. *S. mesogitana* inhibited not only bacterial growth but also the levels of migration of SHSY-5Y cancer cells. These findings indicate that *S. mesogitana* possesses potent antioxidant, anticancer, antimicrobial and antibiofilm activities associated with its bioactive phenolic constituents.

*Keywords*: HPLC; phenolics; antioxidant; cytotoxic; antimigration; antibiofilm.

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## INTRODUCTION

Traditional plant knowledge acquired by a nation for centuries has mostly been passed on to the next generation. This precious knowledge, assembled via ethnobotanical research, is vital for the protection, and for understanding the uses of native and local plants.[1] These days, literature is loaded with accumulating proof highlighting the necessity to incessantly screen medicinal plants to discover key details on their medicinal significance. In fact, within years, the comprehensive growth of nutraceutical, pharmaceutical, cosmeceutical and food industries has dramatically enhanced the requisition for medicinal plants and their bioactive components.[2]

*Saponaria* L. is a genus of flowering plants belonging to a family commonly known as the pink or the carnation family (Caryophyllaceae), which has 81 genera distributed throughout Mediterranean and temperate regions.[3] The genus *Saponaria* consists of about 40 species worldwide. In Turkey, this genus is represented by 20 species, which is the richest diversity in the world.[4] It has been demonstrated that members of this genus have acaricidal, antibacterial, antioxidant and antiproliferative properties.[5–8] *Saponaria* species, commonly known as soapworts, derive their name from their notable high content of triterpene saponins.[9] These species also include fatty acids, quillaic acid, flavonoids and other phenolic compounds in addition to saponins.[10,6] Phenolic compounds commonly found in plants have a wide range of biological activities, including antioxidant properties.

Antioxidants could prevent the oxidation of biomolecules in food as well as in human cells. Industrial antioxidants are mostly synthetic components that could negatively impact human health. Natural antioxidants are crucial in fight against free radicals, which could be related to many diseases such as Alzheimer's disease, atherosclerosis, emphysema and many forms of cancer.[11] Due to their nature, green plants, fruits and vegetables are often used as primary natural antioxidant sources. Phenolic compounds, found in plant extracts, are known to act as strength-free radical scavengers and exhibit antioxidant properties.[12]

Cancer is a significant global health issue, causing 11 million deaths by 2030.[13] Neuroblastoma is one of the most common pediatric cancer and the prognosis is poor. Neuroblastoma treatment involves chemotherapy, surgery, and radiotherapy. However, systemic toxicity in treatment hinders patient well-being, necessitating a new, affordable approach. Medicinal plants are crucial in cancer drug discovery, as they contain notable anti-cancer agents.[14] The cytotoxic effect of several natural compounds and plant extracts has been shown *in vitro* and *in vivo* studies on neuroblastoma cells.[15,16]

Today, while antibiotic resistance rates are rapidly increasing, the investment made by the pharmaceutical industry in antibiotic discovery is gradually decreasing due to the resistance problem. For this reason, the importance of antimic-

robial effective compounds obtained from natural sources in the treatment of infectious diseases is increasing. In infections caused by multidrug-resistant bacteria, one of the most important factors that increase the virulence of these bacteria is the biofilm structure. Bacterial biofilms mediate recurrent infections and antimicrobial resistance through different mechanisms.[17] Evaluating the antimicrobial and antibiofilm effects of compounds with different chemical groups (such as phenolic compounds) isolated from natural sources, especially plants, is critical for treating infectious diseases.[18]

In this context, *Saponaria* species are one of the plants whose antimicrobial effects have been investigated, and the majority of the studies in the literature are on the *Saponaria officinalis* species. However, there is no previously published data about the biological activity of *Saponaria mesogitana*, as well as its phytochemical composition. Therefore, the potential antioxidant, anticancer, antimicrobial and antibiofilm activities of *S. mesogitana*, were investigated in this study.

## EXPERIMENTAL

### *Plant material and extraction*

*S. mesogitana* was collected at the flowering stage in midsummer 2018 from Isparta, Eğirdir-Turkey, and identified (Voucher No: NS 3050) by Prof. Olcay Dusen, Pamukkale University. The aerial parts of the plant were air-dried in shadow, powdered as a fine grain (10 g), and then extracted with 100 mL methanol and water at 45 °C for 6 h in a controlled shaker. At the end of this period, the mixture was filtered and the filtrate was evaporated under reduced pressure at 37 °C using a rotary evaporator. The water in the extract was freeze-dried under a vacuum at −51 °C and the resultant extracts were stored at −20 °C until use. In the case of the water extract, the rotary evaporation step was omitted, and the lyophilization process was applied directly.[19] The dried extracts were weighed to determine the percent of yield. The percentage yield was obtained using this formula: $100W1/W0$, where $W1$ is the final weight of the extract and $W0$ is the initial weight of the sample.

### *Determination of total secondary metabolites amount*

To obtain total levels of phenolic, flavonoid, and saponin contents in the methanol and water extracts, colorimetric assays were used as described in the previous paper.[20] Folin–Ciocalteu, aluminum colorimetric and vanillin sulphuric acid methods were used to detect total phenolic, flavonoid and saponin contents in the extracts, respectively. These contents were expressed as gallic acid (mg GAE $g^{-1}$), quercetin (mg QE $g^{-1}$) and quillaja (mg QAE $g^{-1}$) equivalents, respectively.

### *Phenolic compound characterization by HPLC*

The phenolic constituents of *S. mesogitana* were analyzed using RP-HPLC (Shimadzu, Japan) with separation conducted at 30 °C on a reversed-phase column (250 mm×4.6 mm, 5 μm, Agilent Eclipse XDB C-18) employing a mobile phase consisting of a mixture of acetic acid (3 %) and methanol. The mobile phase was pumped at a flow rate of 0.8 mL $min^{-1}$. The polyphenolic chemicals in the methanol extract of *S. mesogitana* were measured in μg $g^{-1}$ and analyzed using a diode array detector at specific wavelengths. Then, retention time and spectrum matching were combined to identify each target chemical.

*Antioxidant activity*

*DPPH radical scavenging antioxidant activity*. The capacity of the extracts to eliminate the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined with the described method with slight modifications.[21] Various concentrations of the extracts were added to the DPPH solution and the mixture was allowed to incubate for 30 min in the dark at room temperature. After incubation, the absorbances were measured at 517 nm. The synthetic antioxidant, butylated hydroxytoluene (BHT) was used as the positive control. The results were indicated as $IC_{50}$.

β-*Carotene/linoleic acid assay*. The β-carotene test system was used to investigate the extracts' ability to inhibit linoleic acid oxidation with slight modifications to the described method.[21] This method is based on the monitoring of the color opening of *β*-carotene by alkyl peroxides formed by free radical chain reaction by heat and air oxidation of linoleic acid. The results were calculated with the following formula as inhibition rate, %, $100(1 - ((A_C - - A_S)/(A_C^o - A_S^o))$, where $A_C$ and $A_C^o$ were absorbance values initial and final measurement of the control group; $A_S$ and $A_S^o$ were absorbance values of samples or standard, respectively. BHT was used as a standard antioxidant.

*Ferric reducing antioxidant power (FRAP) assay*. This assay was carried out according to Apak *et al.* with slight modifications.[22] The principle of this method is based on the reduction of a Fe(III)–tripyridyltriazine (TPTZ) complex to Fe(II)–TPTZ in the presence of antioxidants. The results measured at 593 nm are given as equivalent to Trolox (mg TE/g extract).

*Cytotoxic activity*

The cytotoxic effect of the aerial parts of *S. mesogitana* methanol extract was tested against human neuroblastoma cancer cell line SHSY-5Y using the MTT assay. SHSY-5Y cells were grown in Dulbecco's Modified Eagle Medium (DMEM) supplemented with L-glutamine, penicillin, streptomycin and 10 % heat-inactivated fetal bovine serum (FBS) in a humidified atmosphere of 5 % $CO_2$ air at 37 °C. SHSY-5Y cells were seeded at a density of $5\times10^3$ cells/well in 96-well plates and incubated overnight for cytotoxicity experiments. After treatment with extracts (1–100 μg $mL^{-1}$), MTT solution was added. The formed formazan crystals were dissolved in DMSO, and then absorbance was measured at 570 nm. The control cells were considered 100 % viable, and the $IC_{50}$ value was calculated.[23]

*Antimigration activity*

The effect of *S. mesogitana* methanol extract on the migration of cells was determined with wound healing migration assay. SHSY-5Y cells were seeded into ($2\times10^5$ cells/well) 6-well plates and after 24 h incubation, wells were scratched straightly with a sterile 200 μL pipette tip. After scratching, cells were washed with serum-free DMEM and treated with $IC_{50}$ concentration (47.68 μg $mL^{-1}$) of the extract. DMEM supplemented with 10 % FBS was used for the control group. Cells were incubated for 24 h at 37 °C in a humidified, 5 % $CO_2$ air and photographed under an inverted phase-contrast microscope (Olympus CKX53, Japan) for the comparison of the cell movements.[24] Quantification of the wound area was measured by using the ImageJ/Fiji program, which is a wound healing size tool, allowing for the measure of the wound area and scratch width in μm.

*Antibacterial activity*

*Test microorganisms and mediums*. Antibacterial activity profiles of the *S. mesogitana* methanol extract against the American Type Culture Collection (ATCC) bacterial strains Gram-negative (*Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853) and Gram-positive (*Staphylococcus aureus* ATCC 29213, *Enterococcus faecalis* ATCC 29212)

were investigated by disc diffusion test and broth microdilution method under the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[25] Bacterial strains were grown on Mueller–Hinton agar (MHA). Mueller–Hinton broth (MHB) and tryptic soy broth with 2 % glucose (TSBG) mediums were used for broth microdilution and biofilm formation experiments, respectively. *E. faecalis* was used as a positive control strain for biofilm quantification experiments.

*Disc diffusion method*. The extract's antibacterial activity was tested using a Kirby–Bauer disc diffusion susceptibility test, with bacterial suspensions prepared from fresh colonies on MHA and adjusted to 0.5 McFarland turbidity using a densitometer device (Biosan, DEN-1). The suspensions were spread onto agar plates using sterile cotton swabs, and 10 µL of the 40 mg $mL^{-1}$ extract was absorbed into sterile blank discs (6 mm, Oxoid), and the discs were placed on the agar plates. The plates were incubated at 37 °C for 24 h, with ciprofloxacin discs used for internal quality control. The inhibition zone diameters (mm) on agar plates were measured for each strain at the end of the incubation period.

*Broth microdilution method*. The minimum inhibitory concentrations (*MIC*s) of the extract against bacterial strains were determined by the broth microdilution method.[25] Bacterial strains were grown on MHA, suspended in sterile saline, and diluted. 50 µL of MHB was added to the first wells of 96-well U-bottom microplates, followed by 50 µL of extract, and serial dilutions were prepared using side wells. Bacterial suspensions (50 µL) were inoculated into microplates and incubated at 37 °C for 24 h. The lowest extract concentration that inhibited bacterial growth was determined as the *MIC* value of the extract.

*Antibiofilm activity*

The antibiofilm effect of the *S. mesogitana* methanol extract was assessed using the spectrophotometric microplate method with crystal violet (CV) staining. First, *E. faecalis* and *S. aureus* were allowed to form mature biofilms on the bottom of the sterile 96-well, F-bottom microplates. TSBG medium (180 µL) and bacterial suspension (20 µL) were added to the wells. The microplates were then incubated at 37 °C for 24 h to enable biofilm formation. Following the aspiration of well contents, 200 µL of extract was added to each well, directly onto the mature bacterial biofilm layer. The microplates were further incubated for 24 h. After the incubation period, the well contents were aspirated, and the microplates underwent CV staining.[26,27] The wells were incubated with 0.1 % CV solution for 15 min, then rinsed with tap water until colorless, dried, and then destained with 200 µL of 95 % ethanol for 15 min.

Spectrophotometric measurements were performed using a microplate reader (CLARIOstar Plus, BMG Labtech, Germany) to obtain the optical density (*OD*) values at 570 nm. To determine the percentages of biofilm inhibition, the *OD* values were used in the following formula:

$$\text{Percentage of biofilm inhibition} = 100(OD_A - OD_B)/OD_A \quad (1)$$

where $OD_A$: the optical density of biofilm control well without plant extract and $OD_B$: the optical density in the presence of the plant extract.

The biofilm production capacities for bacterial isolates were categorized according to the following criteria: $OD \leq ODc$: no biofilm production, $ODc < OD \leq (2ODc)$: weak biofilm producer, $(2ODc) < OD \leq (4ODc)$: moderate biofilm producer and $(4ODc) < OD$: strong biofilm producer.

*Statistical analysis*

Each experiment was done in triplicate. The results obtained are expressed as mean ± standard deviation (*SD*). The $IC_{50}$ values were determined with GraphPad Prism 9 software

(San Diego, CA, USA). Statistical comparisons were performed using an analysis of variance followed by Tukey's post hoc test. The significance was accepted as *$p$<0.05, ****$p$<0.0001.

## RESULTS AND DISCUSSION

The abundance of peer-reviewed studies on herbs that research groups have published demonstrates the extent to which herbal medicine research is being conducted in this day and age. The ongoing search for novel drugs from plants, despite the abundance of research being conducted, remains a crucial necessity.[28]

One of the most important plant genera with traditional folklore importance is *Saponaria*, which makes it a possible source of bioactive chemicals.[29] In this study, the total phenolic content of *S. mesogitana* extracts was calculated as equivalent to gallic acid, and the highest content was observed in methanol extract (32.28 mg GAE $g^{-1}$). Our results showed that methanol extract has the highest total flavonoid amount (24.07 mg QE $g^{-1}$), while water extract has the highest total saponin amount (65.30 mg QAE $g^{-1}$). The yield of extracts from *S. mesogitana* is listed in Table I. The efficiency of extracts prepared with two solvents with different polarities was calculated. The highest extract amount was obtained from water extract (21.08 %). This result can be related to water highest polarity. However, methanol presents a lower extraction yield with a percentage of 14.80 %.

TABLE I. Extract yield and total secondary metabolites amount of *S. mesogitana* (mean ± *SD*); *TPA:* total phenolic amount; *TFA:* total flavonoid amount; *TSA:* total saponin amount; GAE: gallic acid equivalents; QE: quercetin equivalents; QAE: quillaja equivalents, nd: not detected. In each row, different letters indicate a significant difference ($p$<0.05)

| Parameter | Methanol | Water |
|---|---|---|
| Extraction yield, % | 14.80±0.10$^{a}$ | 21.08±018$^{b}$ |
| *TPA* / mg GAE $g^{-1}$ | 32.28±0.21$^{c}$ | 25.30±0.18$^{b}$ |
| *TFA* / mg QE $g^{-1}$ | 24.07±0.20$^{b}$ | 20.15±0.22$^{b}$ |
| *TSA* / mg QAE $g^{-1}$ | 48.12±0.33$^{c}$ | 65.30±0.52$^{d}$ |

To compare the results and produce more accurate data, the antioxidant activity of *S. mesogitana* extracts prepared with methanol and water was assessed using three different methods, including $\beta$-carotene/linoleic acid, DPPH and FRAP assay. The methanolic extract showed a slightly higher antioxidant activity than the aqueous extract in all assays. DPPH radical scavenging activity of the extracts was found to be close to the synthetic antioxidant, BHT. Our results suggested that free radical scavenging activities increased with the phenolic contents of the extract. The total phenolic amount of *Saponaria cypria* methanol extract was reported earlier as 13.62 mg GAE $g^{-1}$.[30] According to this result, *S. mesogitana* methanolic extract had more abundant (32.28 mg GAE $g^{-1}$) total phenolic amounts than *S. cypria.* Phenolic compounds are known to be molecules with antioxidant activity due to their hydroxyl groups and phenolic rings.[31] The antioxidant activity of the extracts is presented in Table II.

TABLE II. Antioxidant activity of *S. mesogitana* extracts (mean ± *SD*); TE: trolox equivalents. In each row, different letters indicate significant differences ($p<0.05$)

| Assay | Methanol | Water | BHT |
|---|---|---|---|
| β-carotene/linoleic acid (inhibition, %) | 74.52±1.44$^{c}$ | 70.24±1.40$^{c}$ | 94.15±1.60$^{d}$ |
| DPPH ($IC_{50}$ / μg mL$^{-1}$) | 11.65±0.09$^{b}$ | 13.44±0.10$^{b}$ | 10.02±0.07$^{a}$ |
| FRAP (mg TE g$^{-1}$) | 83.17±1.61$^{d}$ | 55.31±1.38$^{c}$ | – |

Although numerous studies have focused on the saponins of the genus *Saponaria*, research on its phenolic compounds is quite limited.[10,32] Methanol extract was utilized in HPLC analysis and other bioactivity tests since it has a greater antioxidant capacity and total phenolic content than water. To identify the phenolic compounds in the methanolic extracts of the aerial parts of *S. mesogitana*, 15 standard compounds (gallic acid, 3,4-dihydroxybenzoic acid, 4-hydroxybenzoic acid, 2,5-dihydroxybenzoic acid, chlorogenic acid, vanillic acid, epicatechin, caffeic acid, *p*-coumaric acid, ferulic acid, rutin, ellagic acid, naringin, cinnamic acid, quercetin) were used in the HPLC analysis. The phenolic compounds were detected in the extract with varying amounts and have been listed in Table III. According to these results, caffeic acid (11410.76 μg g$^{-1}$), epicatechin (4394.09 μg g$^{-1}$), 2,5-dihydroxybenzoic acid (4173.63 μg g$^{-1}$) and quercetin (1413.89 μg g$^{-1}$) are most common phenolic compounds of the extract. It has been reported that these phenolics possess antioxidant, anticancer, and antibacterial activities.[33–35]

TABLE III. Phenolic compounds characterization of methanolic *S. mesogitana* extract by HPLC (mean ± *SD*); *RT*: retention time, *LOD*: limit of detection

| No. | Identified phenolic compound | *RT* min | UV $\lambda_{max}$ nm | *LOD* μg mL$^{-1}$ | Content, μg/g extract (mean±*SD*) |
|---|---|---|---|---|---|
| 1 | Gallic acid | 6.8 | 280 | 0.015 | 18.63±0.45 |
| 2 | 3,4-Dihydroxybenzoic acid | 10.7 | 280 | 0.031 | 09.24±0.16 |
| 3 | 4-Hydroxybenzoic acid | 15.7 | 280 | 0.014 | 11.09±0.24 |
| 4 | 2,5-Dihydroxybenzoic acid | 17.2 | 320 | 0.753 | 4173.63±65.47 |
| 5 | Chlorogenic acid | 18.2 | 320 | 0.011 | 146.18±1.83 |
| 6 | Vanillic acid | 19.2 | 320 | 0.112 | 802.52±4.87 |
| 7 | Epicatechin | 21.3 | 260 | 0.433 | 4394.09±67.00 |
| 8 | Caffeic acid | 22.7 | 280 | 0.018 | 11410.76±242.4 |
| 9 | *p*-Coumaric acid | 26.1 | 320 | 0.020 | 31.38±0.26 |
| 10 | Ferulic acid | 30.1 | 320 | 0.012 | 608.71±4.12 |
| 11 | Rutin | 45.6 | 360 | 0.576 | 316.91±3.11 |
| 12 | Ellagic acid | 47.7 | 240 | 0.455 | 607.27±4.06 |
| 13 | Naringin | 49.7 | 280 | 0.404 | 88.22±0.62 |
| 14 | Cinnamic acid | 67.8 | 280 | 0.016 | 59.73±0.50 |
| 15 | Quercetin | 71.1 | 360 | 0.578 | 1413.89±14.74 |

Using herbs and medicinal plants for primary human health care is a universal phenomenon. Today, as much as 80 % of the people in the world depend on traditional medicine as primary health care.[36] Therefore, such plants need to be investigated to understand their chemical constituents and pharmacological activities. *S. mesogitana* is used in folk medicine for kidney stones (as litholytic), joint inflammation, acne, stomach aches and liver diseases.[37] There is no prior research on this plant's ability to fight cancer. To evaluate its anticancer activity, we have chosen SHSY-5Y neuroblastoma cells, which have not previously been studied in the context of this genus. We have selected these cells due to their rapid proliferation, experimental accessibility and established role as a model system in neurodegenerative disease research.[38] MTT assay and wound healing migration assay were used to investigate the plant's anticancer activity. The effect of methanolic extracts of *S. mesogitana* against SHSY-5Y neuroblastoma cells resulted in a concentration-dependent decrease in cell viability. The half--inhibition concentration ($IC_{50}$) value was determined to be 47.68±2.01 μg $mL^{-1}$ (Fig. 1). According to cell migration results, obtained using wound-healing assay, the extract reduced cell migration in SHSY-5Y cells, compared with the control group. 0 and 24 h images were given in Fig. 2. In this assay, it was observed that the control cells migrated faster than the cells treated with the plant extract when both the differences in the wound areas and the width of the scratch were evaluated, as the control groups and *S. mesogitana*-treated groups were compared (Fig. 3). Wound closure percent was calculated as 63 and 28 % in control and extract-treated cells, respectively. When we compared the cell migration rate (μm/h) between extract-treated and non-treated cells, *S. mesogitana*-treated cells had a rate of 3.5 μm/h while non-treated cells had 4.18 μm/h (Fig. 4).

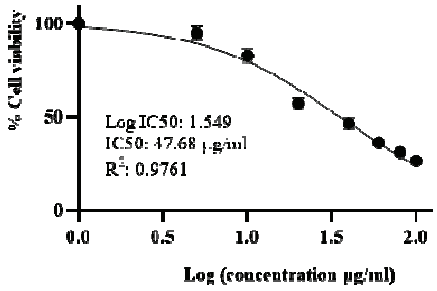


Fig. 1. Percentage cell viability of SHSY-5Y cells treated by *S. mesogitana* extract in different concentrations calculated by MTT assay.

Anticancer activity is attributed to saponins found in the roots of other *Saponaria* species.[10] In this study, the anticancer properties of the extract obtained from the aerial part seem to be related to the phenolic compounds of the plant. We used SHSY-5Y cells to show the effect on the *S. mesoginata* for the first time. We believe that it can be helpful to maintain further studies focused on neuroscience and *S. mesogitana*.

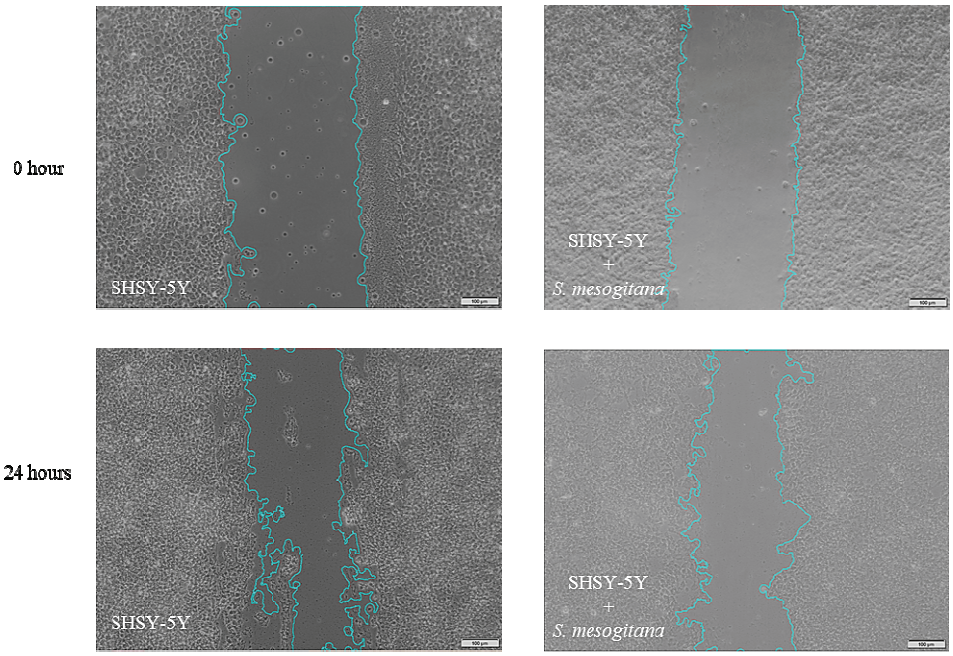


Fig. 2. Wound healing images of SHSY-5Y and SHSY-5Y treated with *S. mesogitana* at 0 and 24 h.

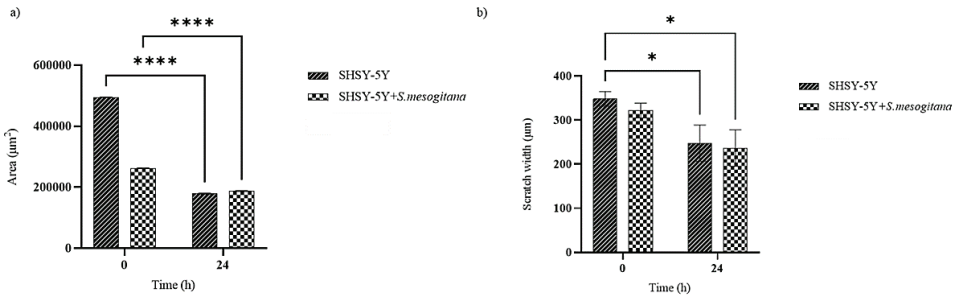


Fig. 3. a) Wound area at time 0 and after 24 h incubation. b) Scratch width at time 0 and after 24 h incubation. *$p$<0.05, ****$p$<0.0001.

In the disk diffusion experiments conducted in this study, 11 and 10 mm zone diameters were measured around the extract-containing disks for *S. aureus* and *E. faecalis*, respectively. No inhibition zone was measured for *P. aeruginosa* and *E. coli* strains in Kirby–Bauer disc diffusion susceptibility test. In the line with these results, the broth microdilution method was applied to determine the minimum inhibitory concentrations of the extract against *S. aureus* and *E. faecalis* strains.

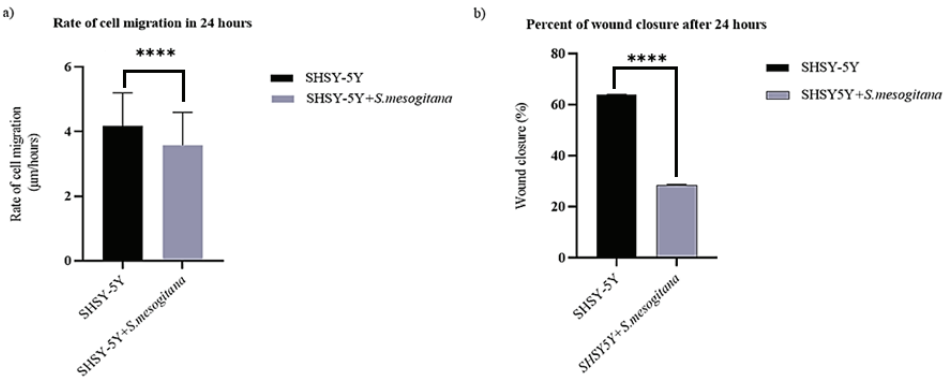


Fig. 4. a) Rate of cell migration in 24 h and b) percent of wound closure. ****$p$<0.0001.

As a result of broth microdilution assays to determine the antimicrobial activity of *S. mesogitana* extract, the minimum inhibitory concentrations against *S. aureus* and *E. faecalis* bacteria were determined as 1.56 and 0.195 mg $mL^{-1}$ (Table IV). Biofilm quantification and antibiofilm activity of the extract were investigated with the spectrophotometric microplate method. It was determined that biofilm production of *S. aureus* and *E. faecalis* decreased by 69.0 and 85.2 %, respectively, in the presence of the extract.

TABLE IV. The minimum inhibitory concentrations (*MIC*s) and antibiofilm effects of *S. mesogitana*; *OD*: optical density on 570 nm

| Bacterial strain | *MIC* mg $mL^{-1}$ | *OD* | Biofilm forming capacity | *OD* in the presence of the extract | Biofilm inhibition, % |
|---|---|---|---|---|---|
| *S. aureus* ATCC 29213 | 1.56 | 0.2443 | Weak biofilm producer | 0.0761 | 69.0 |
| *E. faecalis* ATCC 29212 | 0.195 | 0.4364 | Strong biofilm producer | 0.0645 | 85.2 |

The zone diameter for *S. aureus* in the methanolic extract for *S. officinalis* was reported as 18 mm by Sengul *et al.* while the zone diameter of the methanol extract against the same bacteria was recorded as 11 mm in our study.[7] Eren *et al.* determined the *MIC* value of *S. officinalis* against *E. faecalis* as 8 mg $mL^{-1}$, in our study, the *MIC* value for the methanol extract of *S. mesogitana* species against the same bacterial species was detected as lower than 8 mg/mL.[39]

There are several studies in the literature that draw attention to the antimicrobial effects of different *Saponaria* species, especially focused on *S. officinalis*. Similar to our study, previous studies have examined different gram-positive and gram-negative bacterial species and generally reported higher antimicrobial effects of *Saponaria* extracts against gram-positives. Among them, Charalambous *et al.* conducted with *Saponaria cypria* Boiss. root extracts, the *MIC* values

for *S. cypria* methanol extracts against *S. aureus* and *E. faecalis* were 1.563 and 3.125 mg $mL^{-1}$, respectively.[30] The *MIC* value of *S. mesoginata* species against *E. faecalis* bacteria was much lower in the present study.

The antifungal and antibacterial activities of the *Saponaria prostrata* plant samples against different bacterial and fungal strains were investigated by Aras and Alan.[40] They indicated that the highest antibacterial activity was detected against the *S. aureus* ATCC 25923. It was also reported that the extract did not demonstrate any antimicrobial activity against *E. coli*, *P. aeroginosa*, *C. albicans*, *Y. lipolytica* and *S. cerevicia*. Similarly, we detected no inhibition zone for *S. mesoginata* extract against *P. aeruginosa* and *E. coli* strains in the disk diffusion tests.

To the best of our knowledge, there is no data on the antibiofilm effect of *S. mesogitana*. In this study, in addition to the antimicrobial effect of *S. mesogitana*, its antibiofilm activity against two bacterial strains was also investigated. The effect of *Saponaria* species, including the widely studied *S. officinalis*, on bacterial biofilms is not yet known. Our study is unique in terms of investigating the antibiofilm activity of *S. mesogitana*, and a strong antibiofilm effect was detected on the biofilm structures of *S. aureus* and *E. faecalis* species. In this study, we found that the methanol extract of *S. mesogitana* inhibited biofilm formation at a high level in two tested bacterial strains. The methanol extract of *S. mesogitana* showed an inhibitory effect on *S. aureus* and *E. faecalis* biofilm formation, 80.7 and 85.2 %, respectively (Table IV). These results are promising in terms of benefiting from the potential antibiofilm effect of the extract obtained from *Saponaria* species. Considering that the discovery of new antimicrobial agents is quite limited, and the frequency of infections associated with bacterial biofilms, antibacterial and antibiofilm it is clear that effective extracts/compounds obtained from natural sources are of critical importance.

## CONCLUSION

Phytochemicals, a class of bioactive substances found in medicinal plants, have been utilized extensively worldwide to treat a wide range of diseases. Unlike the genus *Saponaria*, which is known for the saponins it contains, the phenolic constituents of the extract obtained from the aerial parts of *S. mesogitana* species belonging to the same genus were investigated for the first time in this study. Besides its phytochemical composition, various pharmacological effects of *S. mesogitana*, including antioxidant, anticancer, antimicrobial and antibiofilm effects are also firstly explored. It can be suggested that the observed biological activities of the extract are due to the presence of different phenolic compounds. Our results showed that *S. mesogitana* could be accepted as a novel and alternative natural source of antioxidative, antitumoral, and antimicrobial agents. For this reason, this plant may contribute to the development of new drug

or food additive formulations. Additionally, such studies are valuable for uncovering the contents of traditional plants. Despite being the first study on this plant, more *in vitro* and *in vivo* research is required to fully grasp its potential.

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ИЗВОД

## ИСПИТИВАЊЕ БИОАКТИВНОСТИ И ФИТОХЕМИЈСКОГ САСТАВА МЕТАНОЛНОГ ЕКСТРАКТА *Saponaria mesogitana*

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Врста *Saponaria* садржи сапонине који имају различите биолошке активности. Фенолна једињења *S. mesogitana* нису до сада испитана. У овој студији је одређен фенолни састав и биолошка активност једињења из *S. mesogitana*. Одређена је антиоксидативна активност метанолног и воденог екстракта применом DPPH, FRAP и теста на бази *β*-каротена и линолне киселине, као и садржај укупних секундарних метаболита, укључујући фенолна једињења, флавоноиде и сапонине. На основу антиоксидативне активности и садржаја фенолних једињења и флавоноида, изабран је метанолни екстракт за даљу HPLC анализу и експерименте одређивања антитуморске и антимикробне активности. Антитуморски потенцијал је мерен применом MTT и теста миграције код зарастања рана, а антибактеријски применом теста дифузије на диску и одређивањем *MIC*. Способност екстракта да спречи формирање биофилма одређивана је кристал виолет методом. Метанолни екстракт је испољио велику антиоксидативну активност, а кафеинска киселина и епикатехин су главна фенолна једињења идентификована HPLC методом. *S. mesogitana* је способна да инхибира бактеријски раст, као и миграцију канцерских ћелија SHSY-5Y. Добијени резултати показују да *S. mesogitana* има јаку антиоксидативну, антиканцерску, антимикробну активност и да инхибира стварање биофилма, захваљујући својим фенолним једињењима.

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