

Two Unexplored *Saponaria* Species from Türkiye: Phenolic Composition and a Pharmacologic Potential

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Received: 11-18-2024

Abstract

The total secondary metabolite amounts, phenolic profile, antioxidant activity, acute toxicity, cytotoxic, and wound-healing effects of the aerial parts of *Saponaria kotschy* (endemic) and *Saponaria pumilio* extracts were investigated. The total phenolic, flavonoid, saponin, and tannin contents; antioxidant activity (via six different methods, including β -carotene/linoleic acid, DPPH, ABTS, FRAP, phosphomolybdenum, and metal chelating assays); phenolic constituents in the extracts (HPLC method); acute toxicity (brine shrimp lethality test); antiproliferative effects on human cervical carcinoma (HeLa) and human neuroblastoma (SH-SY5Y) cancer cell lines (MTT assay); and the effects on wound healing in healthy mouse fibroblast cells (NIH-3T3) through an in vitro scratch assay were evaluated. The methanol extract of *S. kotschy* demonstrated higher concentrations of secondary metabolites and antioxidant activity than *S. pumilio*. Both species predominantly contained caffeic acid, 2,5-dihydroxybenzoic acid, and epicatechin, though *S. pumilio* had a notably higher caffeic acid content. Additionally, *S. kotschy* extract showed greater cytotoxicity against HeLa and SH-SY5Y cancer cells, while *S. pumilio* exhibited greater wound-healing efficacy. Two previously unexplored *Saponaria* species reveal a remarkable richness in secondary metabolites and potent antioxidant activity. Given their diverse phenolic constituents, these species emerge as promising candidates for therapeutic applications in cervical cancer and neuroblastoma, as well as playing a potentially critical role in tissue repair and regeneration-areas that merit further in-depth investigation.

Keywords: *Saponaria*; HPLC analysis; phenolic composition; wound healing; anticancer

1. Introduction

The genus *Saponaria*, belonging to the Caryophyllaceae family, shows the greatest biodiversity in the world in Türkiye. Due to its unique geographical location at the Mediterranean, Asia, and Europe intersection, Türkiye is home to a rich diversity of plant species and numerous endemic plants, influenced by various climatic zones.¹ For thousands of years, people have used plants as food, medicine, handicrafts, and decoration. Approximately 80% of the world's population uses alternative medicine as their major source of healthcare, and the usage of herbal products in treating various diseases is on the rise.² Therefore, systematically analyzing plants and their derived products

used in alternative medicine across various parameters is becoming increasingly important.³

This genus is represented by 20 species and 23 taxa in Türkiye, 11 of which are endemic.⁴ Commonly referred to as soapworts, *Saponaria* species are perennial, blooming plants that are native to Europe and Asia.⁵ As their common names suggest, due to the well-documented high content of triterpene saponins in their roots, they can be used as a gentle liquid soap by boiling the roots in water.⁶ In addition to saponins, soapworts contain flavonoids, phenolic compounds, and fatty acids.^{7,8} Members of this genus have been shown to possess antioxidant, antimicrobial, antiscrophulatic, and antiproliferative activities. In addition, they are used for acne, stomach aches,

liver diseases, stones in the kidney, and joint inflammation.^{9,10}

The body's defense mechanisms rely on antioxidant enzymes to maintain the critical balance between oxidants and antioxidants. Additionally, plant-derived antioxidants have shown effectiveness against degenerative diseases linked to oxidative stress.¹¹ This has led to growing interest in studying the impact of therapeutically beneficial plants on oxidative damage. Phenolic compounds, secondary metabolites commonly found in plants, exhibit a wide range of biological activities. Notably, they possess strong antioxidant properties, neutralizing free radicals and reducing oxidative stress, which helps prevent cellular damage. Many phenolics also demonstrate cytotoxic effects, inhibiting the growth of abnormal cells, such as cancer cells, thus showing potential in cancer therapy. Additionally, these compounds promote wound healing by reducing inflammation and enhancing tissue regeneration. Overall, phenolic compounds play a significant role in health maintenance and disease management.¹²

In the last few decades, several biological investigations have been conducted on extracts and bioactive phytochemicals, especially saponins, from the plant species *Saponaria*.¹³ On the other hand, the available literature shows a lack of information on the pharmacological properties and chemical composition of *Saponaria kotschyi* Boiss. and *Saponaria pumilio* Boiss., called 'yar sabunu' and 'zarif sabunu' in Turkish. In this sense, the current work attempted to investigate the total secondary metabolites amounts, phenolic profile, antioxidant activity, acute

toxicity, cytotoxic, and wound-healing effects of the aerial parts of *S. kotschyi* and *S. pumilio* for the first time.

Based on the rich phenolic profiles and traditional medicinal uses of *Saponaria* species, we hypothesize that the methanol extracts of *S. kotschyi* and *S. pumilio* will exhibit superior pharmacological activities compared to their water extracts. These activities are expected to include significant cytotoxic effects against cancer cells, enhanced wound healing efficacy, and potent antioxidant properties, driven by their phenolic compound composition.

2. Materials and Methods

2.1. Plant Materials and Extraction Procedure

S. kotschyi and *S. pumilio* were collected in 2018 from around Salda Lake, Burdur-Turkey (1140 m), and Akseki, Antalya-Türkiye (1100 m), respectively (Figure 1), and authenticated by Prof. Olcay Dusen in the Department of Biology, Pamukkale University. The plants were collected during their flowering period in May and June.

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.¹⁴ The dried extracts were weighed to determine the percent of yield. The percentage yield was obtained using this formula: $W1/W0 \times 100$, where W1 is the final weight of the extract and W0 is the initial weight of the sample.

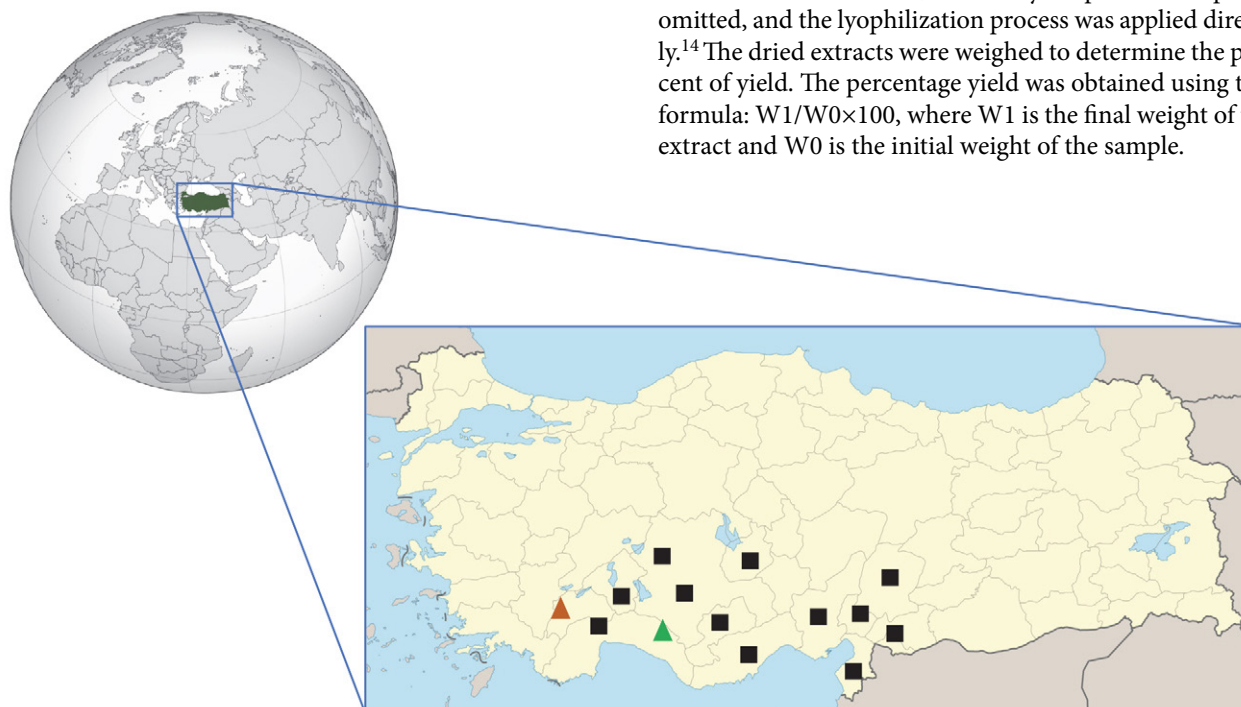


Figure 1. The map of the distribution of *S. kotschyi* and *S. pumilio*. (The black square shows the distribution of both plants, while the red and green triangles mark the collection sites of *S. kotschyi* and *S. pumilio*, respectively).

2. 2. Determination of Total Secondary Metabolites Amount

The total phenolic, flavonoid, saponin, and tannin amounts in the methanol and water extracts were detected using the Folin-Ciocalteu, aluminum, and vanillin sulphuric acid methods, respectively, and expressed as gallic acid (mgGAE/g), quercetin (mgQE/g), and quillaja (mgQAE/g) equivalents, as described in the previous paper.¹⁵ The total tannin amount of the extracts was determined using Bekir *et al.*¹⁶'s method and expressed as catechin equivalents (mgCE/g).

2. 3. Phenolic Compound Characterization by HPLC

The phenolic constituents of the methanolic extracts of *S. kotschyi* and *S. pumilio* were analyzed using RP-HPLC (Shimadzu, Japan) with separation performed at 30 °C on a reversed-phase column (250 mm × 4.6 mm, 5 µm, Agilent Eclipse XDB C-18). The mobile phase consisted of 3% acetic acid and methanol, pumped at a flow rate of 0.8 mL/min. Polyphenolic compounds in the extracts were quantified in µg/g and detected using a diode array detector at specific wavelengths. Retention time and spectrum matching were used to identify each target compound.

2. 4. Antioxidant Capacity Assays

2. 4. 1. DPPH Radical Scavenging Assay

The capacity of the extracts to eliminate the 1,1-diphenyl-2-picrylhydrazyl (DPPH) radical was determined with the described method with slight modifications.³ 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 IC₅₀.

2. 4. 2. ABTS Radical Cation Assay

The ABTS assay is based on the generation of a blue/green 2,2'-azino-bis (3-ethylbenzothiazoline-6 sulfonic acid) (ABTS) radical cation that can be reduced by antioxidants. ABTS radical scavenging activity was analyzed as described by Re *et al.*¹⁷ with some modifications. Freshly prepared and diluted ABTS solution were mixed with the various solvent extracts of the plants and the absorbances were read after 30 min at 734 nm. BHT was used as a positive control. The results were indicated as IC₅₀.

2. 4. 3. 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.³ This method is based on monitoring the color opening of β-carotene by alkyl peroxides, which are formed through a free radical chain reaction initiated by heat and air oxidation of linoleic acid. The results were calculated with the following formula as inhibition rate, %; $[1 - (A_C - A_S / A_C^0 - A_S^0)] \times 100$, where A_C and A_C^0 were absorbance values initial and final measurement of the control group; A_S and A_S^0 were absorbance values of samples or standard, respectively. BHT was used as a standard antioxidant.

2. 4. 4. Ferric Reducing Antioxidant Power (FRAP) Assay

This assay was carried out according to Apak *et al.*¹⁸ with slight modifications. 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).

2. 4. 5. Phosphomolybdenum (PM) Assay

This assay is based on the reduction of Mo(IV) to Mo(V) with antioxidant agents. The green color resulting from the reduction is measured at 695 nm. PM assay of the plant extracts carried out Prieto *et al.*¹⁹ Results are given as ascorbic acid equivalents (mg AE/g extract).

2. 4. 6. Metal Chelating Assay

The ferrous chelating capacity of the plant extracts was determined with method by Dinis *et al.*²⁰ with slight modification. According to this method, extracts inhibit ferrozine complexing with Fe²⁺ and color expansion is determined spectrophotometrically (562 nm). The results were given as ethylenediaminetetraacetic acid (EDTA) equivalents (mg EDTAE/g extract).

2. 5. Brine Shrimp Lethality Test

Brine shrimp (*Artemia salina* L.) lethality test (BSLT) was performed as previously reported.¹⁴ Experiments were conducted alongside a control group, with five different extract concentrations (10–1000 µg/mL), each tested in a set of three tubes. Briefly, *A. salina* eggs were incubated in seawater at 28°C for 48 hours to reach the nauplii stage. The nauplii were then transferred to tubes containing the brine solution with the dissolved extracts and allowed to breed for 24 hours in the presence of these concentrations. Following the extract incubation, the number of shrimp that survived was counted, and their vitality was assessed to that of the untreated control group. The cytotoxic drug, etoposide was used as positive control. Data were analyzed using the EPA Probit Analysis program to determine LC₅₀ values.

2. 6. Cell Culture Assays

2. 6. 1. Cell Viability Assay

The human neuroblastoma (SH-SY5Y), human cervical carcinoma (HeLa) and embryonic mouse fibroblast (NIH-3T3) cell lines were maintained in DMEM medium and the cell viability was detected based on the MTT assay as previously described in detail.²¹ The cells were seeded in 96-well plates at a density of 1×10^4 cells/well and incubated overnight. The next day, the cells were treated with methanolic extracts of the plants (100 µg/mL) for 24 hours, followed by the addition of MTT solution. Formazan formation was spectrophotometrically quantified at 520 and 620 nm using a microplate reader. Cell viability was calculated and revealed as a percentage (%) of control. Afterwards, graphics were created.

2. 6. 2. Wound Healing Migration Assay

The wound healing assay is based on observing cell migration into a 'wound' created in a cell monolayer. The rate of wound closure and cell migration is subsequently quantified through photography using an inverted light microscope.²² For the *in vitro* wound healing assessment of the methanolic extracts of the plants, NIH-3T3 fibroblast cells were seeded into (5×10^4 cells/well) 12-well plates and after 24h incubation, wells were scratched straightly with a sterile 200 µL pipette tip. After scratching cells were washed with PBS and treated with various concentrations (1, 10, 50 µg/mL) of the extracts for 18h. Cell migration was observed under an inverted phase-contrast microscope. Data were analyzed using the ImageJ software and calculations for the wound closure percentage (WC%) were performed using the following equation: $WC(\%) = [(A_0) - A(t)/A_0] \times 100$ where A_0 is the area at time zero (0) and $A(t)$ is the area after incubation time (t).

2. 7. Statistical Analysis

Statistical analysis and data processing were performed by using GraphPad Prism 8.0.2. Comparisons of the treatments among groups were analyzed by one-way ANOVA with post-hoc Tukey's test. The significance was

accepted as $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, $****p < 0.0001$.

3. Results and Discussion

3. 1. Extract Yield and Total Secondary Metabolites Amount

S. kotschyi and *S. pumilio* aerial samples were extracted with methanol and water, and the extract yield was calculated. In both species, the methanol extracts yielded higher results compared to water extracts. The highest extraction efficiency was observed in the methanol extract of *S. kotschyi*, with a yield of 35.23% (Table 1).

The higher extract yield and concentrations of secondary metabolites obtained with methanol can be attributed to its superior solvent properties compared to water. As a polar solvent, methanol is more effective at dissolving a broad range of bioactive compounds, such as flavonoids, phenolics, and saponins, which are common in plant materials. Its polarity allows for the extraction of both polar and moderately non-polar compounds, enhancing the recovery of complex phytochemicals often found in glycosylated or conjugated forms.²³

Despite the vast array of bioactive compounds in plants, only about 15% of their phytochemical content has been thoroughly examined, with biological activity screening completed for approximately 6% of these compounds.²⁴ This highlights a significant gap in our understanding of the potential health benefits that remain untapped within plant species. *Saponaria* is a highly significant plant genus with a long history of folklore, which suggests that it may contain bioactive phytochemicals.²⁵

In this study, the total amounts of phenolics, flavonoids, saponins, and tannins in the methanol and water extracts of *S. kotschyi* and *S. pumilio* were quantified using gallic acid, quercetin, quillaja, and catechin as reference standards. The methanol extract of *S. kotschyi* exhibited higher concentrations of all these secondary metabolites compared to the methanol extract of *S. pumilio*, while the water extracts of both species yielded lower values than their respective methanol extracts (Table 1).

Table 1. Extract yield and total secondary metabolites amount of *S. kotschyi* and *S. pumilio* according to different solvents (mean $\pm$ SD).

Plants	Solvents	Extraction yield (%)	TPA (mgGAE/g)	TFA (mgQE/g)	TSA (mgQAE/g)	TTA (mgCE/g)
<i>S. kotschyi</i>	Methanol	35.23 $\pm$ 0.41 ^b	48.43 $\pm$ 0.61 ^b	20.01 $\pm$ 0.25 ^a	16.51 $\pm$ 0.25 ^a	11.03 $\pm$ 0.15 ^a
	Water	26.62 $\pm$ 0.37 ^a	10.03 $\pm$ 1.20 ^a	14.47 $\pm$ 0.18 ^a	10.50 $\pm$ 0.11 ^a	5.17 $\pm$ 0.06 ^a
<i>S. pumilio</i>	Methanol	28.05 $\pm$ 0.40 ^b	34.17 $\pm$ 0.52 ^b	17.55 $\pm$ 0.27 ^a	12.94 $\pm$ 0.14 ^a	6.09 $\pm$ 0.10 ^a
	Water	16.10 $\pm$ 0.22 ^a	11.65 $\pm$ 1.12 ^a	13.06 $\pm$ 0.10 ^a	7.71 $\pm$ 0.08 ^a	3.41 $\pm$ 0.08 ^a

TPA: total phenolic amount, TFA: total flavonoid amount, TSA: total saponin amount, TTA: total tannin amount, GAE: gallic acid equivalent, QE: quercetin equivalent, QAE: quillaja equivalent, CE: catechin equivalent. In each column, different letters indicate a significant difference ($p < 0.05$)

Several studies have investigated the secondary metabolites in *Saponaria* species, with particular emphasis on *S. officinalis*, which primarily contains saponins and phenolic compounds in its roots. The total phenolic amount in the methanolic root extract of *S. officinalis* has been reported as 0.159 mg GAE/g²⁶, whereas *S. cypria* exhibited a higher phenolic amount of 13.62 mg GAE/g in its root extract[8]. Notably, the methanolic extracts from the aerial parts of *S. kotschyi* and *S. pumilio* contained higher total phenolic amounts compared to the roots of *S. cypria* and *S. officinalis*, suggesting that the aerial parts may offer a richer source of phenolics in these species.

3. 2. HPLC Analysis of Phenolic Constituents

To identify the phenolic compounds in the methanolic extracts of the aerial parts of both *S. kotschyi* and *S. pumilio* using 15 standard phenolic 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 extracts in varying amounts and are listed in Table 2. In *S. kotschyi* methanolic extract, the most abundant phenolic compound was 2,5-dihydroxybenzoic acid (4192.72 µg/g), followed by caffeic acid (3377.46 µg/g) and epicatechin (2917.87 µg/g). In contrast, *S. pumilio* exhibited a higher concentration of caffeic acid (10564.70 µg/g) as the dominant phenolic, with 2,5-dihydroxybenzoic acid (5213.87 µg/g), epicatechin (3063.80 µg/g), and quercetin (1101.96 µg/g) following in decreasing abundance.

These results highlight the differences in phenolic composition between the two species. While both species shared caffeic acid, 2,5-dihydroxybenzoic acid, and epicat-

echin as dominant compounds, *S. pumilio* exhibited a significantly higher level of caffeic acid than *S. kotschyi*. Additionally, *S. pumilio* exhibited higher concentrations of quercetin compared to *S. kotschyi*, where it was present in much lower amounts (Table 2).

Research on phenolic compounds in the genus *Saponaria* is limited, as most studies focus on saponins.^{27,7} Methanol extract, which has higher total phenolic and flavonoid content than water, was used for HPLC analysis. Variations in phenolic profiles between *S. kotschyi* and *S. pumilio* may stem from species-specific metabolic pathways as well as environmental factors like soil composition, climate, and geography, which influence secondary metabolite expression. Altitude, temperature, and sunlight exposure further affect phenolic concentrations as plants adjust their chemical profiles in response to external stressors.²⁸

While the phenolic composition of the extracts was characterized using HPLC, the identification relied solely on retention times and spectrum matching with standard compounds. This approach, although effective for preliminary profiling, is limited by the potential co-elution of structurally similar compounds with overlapping retention times. To address this limitation, future studies should incorporate advanced techniques such as LC-MS/MS, which would enable more precise identification and quantification of the bioactive compounds responsible for the observed effects.

Phenolic compound analysis by HPLC has not previously been reported for *S. kotschyi* and *S. pumilio*. However, in the roots of *S. officinalis*, six phenolic compounds—rutin, quercetin galactoside, syringic acid, apigenin, protocatechuic acid, and vanillic acid—were identified in concentrations ranging from 0.010% to 0.069% (*g per 100 g dry root*).²⁶ Additionally, in ethanolic leaf extracts of *S. prostrata*, rutin (36.7 µg/g extract) and hesperidin (32.3 µg/g

Table 2. Characterization of phenolic compounds in methanolic extracts of *S. kotschyi* and *S. pumilio* using HPLC (mean ± SD).

No	Identified phenolic compounds	RT (min)	UV _{max} (nm)	LOD (µg/mL)	<i>S. kotschyi</i> (µg/g extract)	<i>S. pumilio</i> (µg/g extract)
1	Gallic acid	6.8	280	0.015	08.8 ± 2.1	61.2 ± 2.1
2	3,4-dihydroxybenzoic acid	10.7	280	0.031	05.3 ± 2.0	20.2 ± 0.4
3	4-hydroxybenzoic acid	15.7	280	0.014	38.3 ± 11.8	22.7 ± 0.5
4	2,5-dihydroxybenzoic acid	17.2	320	0.753	4192.7 ± 5.1	5213.9 ± 98.5
5	Chlorogenic acid	18.2	320	0.011	379.9 ± 3.0	71.7 ± 2.9
6	Vanillic acid	19.2	320	0.112	648.1 ± 5.4	398.8 ± 3.0
7	Epicatechin	21.3	260	0.433	2917.9 ± 3.3	3063.8 ± 48.1
8	Caffeic acid	22.7	280	0.018	3377.5 ± 26.1	10564.7 ± 21.5
9	<i>p</i> -coumaric acid	26.1	320	0.020	15.8 ± 0.1	12.8 ± 0.3
10	Ferulic acid	30.1	320	0.012	97.9 ± 0.5	05.0 ± 0.0
11	Rutin	45.6	360	0.576	391.6 ± 0.3	448.6 ± 2.8
12	Ellagic acid	47.7	240	0.455	411.0 ± 3.8	697.5 ± 3.8
13	Naringin	49.7	280	0.404	33.2 ± 0.4	20.0 ± 0.8
14	Cinnamic acid	67.8	280	0.016	52.7 ± 4.0	169.7 ± 2.6
15	Quercetin	71.1	360	0.578	230.4 ± 1.4	1101.9 ± 11.0

extract) were identified as the major phenolic compounds using HPLC.²⁹ These values were lower than those obtained in our study from the analyzed *Saponaria* species.

3. 3. Antioxidant Activity

To enable a comparison of the results and obtain more precise data, the antioxidant activity of methanol and water extracts of *S. kotschy* and *S. pumilio* was evaluated using six distinct methods: β -carotene/linoleic acid, DPPH, ABTS, FRAP, phosphomolybdenum, and metal chelating assays (Table 3). Utilizing the β -carotene test system, the extracts' capacity to prevent oxidation of linoleic acid was examined. 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 methanol extract of *S. kotschy* demonstrated the highest antioxidant activity at 87.15%, closely followed by the methanol extract of *S. pumilio*, which exhibited an activity of 84.09%. Similar results were obtained using the phosphomolybdenum assay, a method for measuring total antioxidant capacity, like the β -carotene test system (Table 3).

DPPH and ABTS are free radicals commonly used to assess radical scavenging activities in plant extracts. In these assays, the radicals react with an antioxidant, reducing to non-radical forms. Changes are measured spectrophotometrically, and half-maximal inhibitory concentration (IC_{50}) values are determined. Lower IC_{50} values indicate stronger antioxidant activity. As shown in Table 3, both *S. kotschy* and *S. pumilio* methanol extracts exhibited higher radical scavenging activity in DPPH and ABTS assays compared to their water extracts. In both plants, the ferrous ion-chelating capacity of the extracts was evaluated and calculated as EDTA equivalents. According to the results, the methanolic extracts of both plants exhibited higher chelating capacity than the aqueous extracts.

The FRAP assay is a commonly used method to assess reducing antioxidant capacity. In this assay, antioxidant compounds convert ferric (Fe^{3+}) ions to ferrous (Fe^{2+}) ions. As shown in Table 3, the reducing ability of the water extracts was lower compared to the methanol extracts in both plants.

Medicinal plants are valuable resources for healthcare, particularly in addressing oxidative stress-related conditions. Oxidative stress, an imbalance between free radicals and antioxidants, contributes to chronic diseases such as cardiovascular and neurodegenerative disorders, diabetes, and cancer. Phenolics, flavonoids, and other bioactive compounds in medicinal plants help neutralize free radicals, lowering the risk of these conditions.³⁰

Research on the *Saponaria* genus indicates notable antioxidant activity in various plant parts. For instance, *S. officinalis* aerial parts methanolic extracts demonstrated 70% antioxidant activity using the β -carotene-linoleic acid assay.⁹ In another study, *S. prostrata* ethanol extracts exhibited higher antioxidant activity than water extracts in DPPH and FRAP assays [29]. In this study, methanol extracts consistently outperformed water extracts in antioxidant assays, likely due to methanol's effective extraction of phenolic compounds by disrupting plant cell walls and enhancing release.³¹ The correlation between total phenolic content and antioxidant activity is well-documented; higher phenolic concentrations generally correspond to stronger antioxidant properties.^{32,3} Aligned with these observations, while *S. kotschy* extracts exhibited marginally higher antioxidant activity than *S. pumilio* in most assays, the differences were predominantly subtle, suggesting that phenolic content may play a limited role in influencing antioxidant efficacy under these conditions.

3. 4. BSLT Assay

The brine shrimp lethality test (BSLT) is a simple and high-throughput cytotoxicity test commonly used to assess bioactive compounds, toxins, and herbal extracts. This method evaluates the lethality of test compounds on a basic zoological model, the brine shrimp (*A. salina* L.). BSLT is considered a reliable approach for determining the cytotoxic potential of plant extracts.³³

Table 4 summarizes the acute toxicity results of the plant extracts. The lethality percentage was calculated based on the mean number of dead shrimp larvae in the extract-treated tubes compared to the control. As expected, the lethality was directly proportional to the extract

Table 3. Antioxidant activity of *S. kotschy* and *S. pumilio* extracts (mean $\pm$ SD).

Plants	Solvents	DPPH assay (IC_{50} , μ g/mL)	ABTS assay (IC_{50} , μ g/mL)	β -carotene assay (% inhibition)	FRAP assay (mg TE/g)	PM assay (mg AE/g)	Metal chelating activity (mg EDTAE/g)
<i>S. kotschy</i>	Methanol	12.23 $\pm$ 0.07 ^a	34.12 $\pm$ 0.44 ^a	87.15 $\pm$ 0.23 ^b	77.21 $\pm$ 1.51 ^b	88.43 $\pm$ 1.58 ^b	24.43 $\pm$ 0.32 ^b
	Water	14.76 $\pm$ 0.08 ^a	36.10 $\pm$ 0.47 ^a	78.11 $\pm$ 0.12 ^a	46.18 $\pm$ 0.35 ^a	33.09 $\pm$ 0.41 ^a	18.30 $\pm$ 0.15 ^a
<i>S. pumilio</i>	Methanol	15.88 $\pm$ 0.09 ^a	38.17 $\pm$ 0.51 ^a	84.09 $\pm$ 0.20 ^b	70.62 $\pm$ 0.65 ^b	64.25 $\pm$ 0.73 ^b	22.15 $\pm$ 0.28 ^b
	Water	18.35 $\pm$ 0.13 ^a	40.95 $\pm$ 0.55 ^a	71.54 $\pm$ 0.11 ^a	44.19 $\pm$ 0.65 ^a	31.56 $\pm$ 0.38 ^a	16.09 $\pm$ 0.14 ^a

TE: trolox equivalent, AE: ascorbic acid equivalent, EDTAE: EDTA equivalent. In each column, different letters indicate a significant difference ($p < 0.05$)

concentration, indicating that higher concentrations increased mortality.

For *S. kotschyi*, the methanol extract exhibited a median lethal concentration (LC₅₀) of 6.49 ppm, while the water extract had a significantly lower LC₅₀ of 2.80 ppm, indicating higher toxicity; in comparison, the methanol extract of *S. pumilio* caused a more toxic effect (LC₅₀: 1.55 ppm) and was generally found to be more toxic than *S. kotschyi*.

The higher toxicity of the water extract in *S. pumilio* and the methanol extract in *S. kotschyi* could be attributed to differences in the concentration and types of secondary metabolites within each plant. Environmental factors, such as the distinct growing conditions and habitats of these plants, likely contribute to these variations.³ HPLC analysis identifies both the diversity and concentration of individual phenolic compounds, but this does not necessarily indicate a higher total phenolic content. Total phenolics are typically measured by collectively assessing all phenolic compounds and comparing them against a standard. A plant may possess greater phenolic diversity, but if the concentrations of these compounds are low, the total phenolic content may decrease accordingly. Thus, *S. pumilio* may contain a wider variety of phenolics, but the concentration of each might be lower compared to *S. kotschyi*.

Table 4. The effects of *S. kotschyi* and *S. pumilio* extracts on the death (%) of brine shrimps (mean ± SD).

Concentrations (ppm)	<i>S. kotschyi</i>		<i>S. pumilio</i>	
	Methanol	Water	Methanol	Water
10	66.66	75	77.51	75
50	72.16	80.50	80.50	83.33
100	86.08	86.08	88.83	80.53
500	100	100	100	100
1000	100	100	100	100
Control (dH ₂ O)	27.06	26.45	27.75	26.40
LC ₅₀ (min)	1.42	0.17	0.02	0.12
LC ₅₀	6.49	2.80	1.55	2.54
LC ₅₀ (max)	13.79	8.27	6.34	8.02
LC ₉₀	122.91	83.80	86.33	96.97

3. 5. Cell Viability Assay

Cancer remains a significant global health challenge, largely due to the lack of standardized treatments and the severe side effects associated with chemotherapy. In this context, plants and plant-derived compounds are regarded as valuable resources for discovering novel and alternative therapeutic approaches.³⁴

The anticancer potential of *Saponaria* species has been demonstrated in several studies. For instance, the total methanolic extracts of *S. vaccaria* seeds were evaluated for their growth inhibitory effects in WiDr (colon), MDA-

MB-231 (breast), NCI-417 (lung), and PC-3 (prostate) human cancer cell lines.³⁵ In another study, the inhibitory effects of the methanol:dichloromethane extract of *S. prostrata* subsp. *anatolica* were assessed. The half maximal inhibitory concentration (IC₅₀) of the extract was found to be 259.79 µg/mL in MCF-7 breast cancer cells and 97.24 µg/mL in HCT116 colon cancer cells.³⁶

The potential cytotoxic effects of methanolic extracts obtained from *S. kotschyi* and *S. pumilio* plants were tested on immortalized human cervical cancer (HeLa) and human neuroblastoma (SH-SY5Y) cancer cell lines. As a control, the extracts were also tested on healthy mouse fibroblast cells (NIH-3T3) simultaneously. After incubating the cells with extracts at a 100 µg/mL concentration for 24 hours, cell viabilities were determined using the MTT method. The findings in Figure 2 detail the cell viability levels as a percentage compared to control cells.

Treatment with *S. kotschyi* extract (100 µg/mL) for 24 hours significantly caused cytotoxicity on HeLa and SH-SY5Y cancer cells (54.51% ± 3.79 and 34.03 ± 4.76, respectively) ($p < 0.001$, $p < 0.0001$). Similarly, it was observed to decrease cell viability in healthy NIH-3T3 cells compared to control (19.09 ± 1.05) ($p < 0.0001$).

Likewise, *S. pumilio* extract (100 µg/mL) for 24 hours significantly decreased cell viability on HeLa and SH-SY5Y cancer cells (80.47% ± 1.96 and 69.32 ± 2.84, respectively) ($p < 0.05$, $p < 0.01$). Similar to *S. pumilio* extract, *S. kotschyi* extract also exhibited significant toxic effects on healthy fibroblast (NIH-3T3) cells compared to control cells (66.39% ± 0.93) ($p < 0.01$). Furthermore, *S. kotschyi* extract had higher cytotoxic activity on HeLa and SH-SY5Y cancer cells than *S. pumilio* extract. However, their cytotoxic effects on NIH-3T3 cells used as a control were also high. While *S. kotschyi* extracts demonstrated significant cytotoxic effects on cancer cell lines (HeLa and SH-SY5Y), they also showed pronounced toxicity toward healthy fibroblast cells (NIH-3T3), raising safety concerns for potential ther-

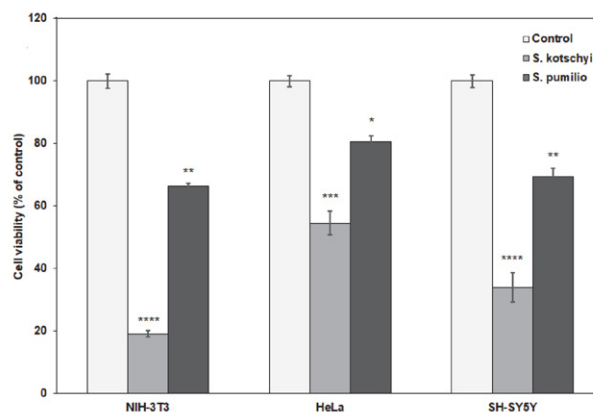


Figure 2. The effects of *S. kotschyi* and *S. pumilio* methanolic extracts on NIH-3T3, HeLa and SH-SY5Y cells viability.

Data are presented as mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

apeutic applications. In contrast, *S. pumilio* extracts exhibited comparatively lower toxicity toward NIH-3T3 cells, suggesting a better safety profile. The observed toxicity of *S. kotschyi* highlights the need for further research to optimize dosing and improve selectivity toward malignant cells while minimizing harm to normal tissues. Strategies such as targeted delivery systems or structural modifications of bioactive compounds could enhance its clinical utility.

This study represents the first investigation into the anticancer potential of *S. kotschyi* and *S. pumilio*. To evaluate their efficacy, we selected SH-SY5Y neuroblastoma cells and HeLa cervical cancer cells as experimental models. Notably, these cell lines have not been previously examined concerning the anticancer properties of this genus, offering a novel perspective for exploring their therapeutic potential.

3. 6. Wound Healing Assay

Alternative medicine for wound care has gained traction, as multicomponent traditional therapies often of-

fer more benefits than single-component allopathic treatments.³⁷ Wound healing is a complex process, and integrating medicinal plants into modern therapies has become a key focus.³⁸ Natural compounds in plants, such as anti-inflammatory and antimicrobial phytochemicals, are widely used due to their affordability and accessibility. However, despite advances in extraction and purification, the mechanisms, side effects, and safety profiles of many compounds remain underexplored.³⁹

The methanolic extracts of *S. kotschyi* and *S. pumilio* were investigated for their effects on wound healing through an *in vitro* scratch assay using embryonic mouse fibroblast cells (NIH-3T3). For this purpose, after creating a standard scratch with a 200 μ L pipette tip, the tested extracts were added into a culture medium at 0, 1, 10 and 50 μ g/mL concentrations and then incubated for 18 hours. The cells were photographed under an inverted microscope at the experiment's beginning and the 18th hour. The photos related to the wound healing experiment, along with the wound closure percentages, are presented in Figure 3 and Table 5, respectively. According to these results, the wound closure in the control group was 56.75%

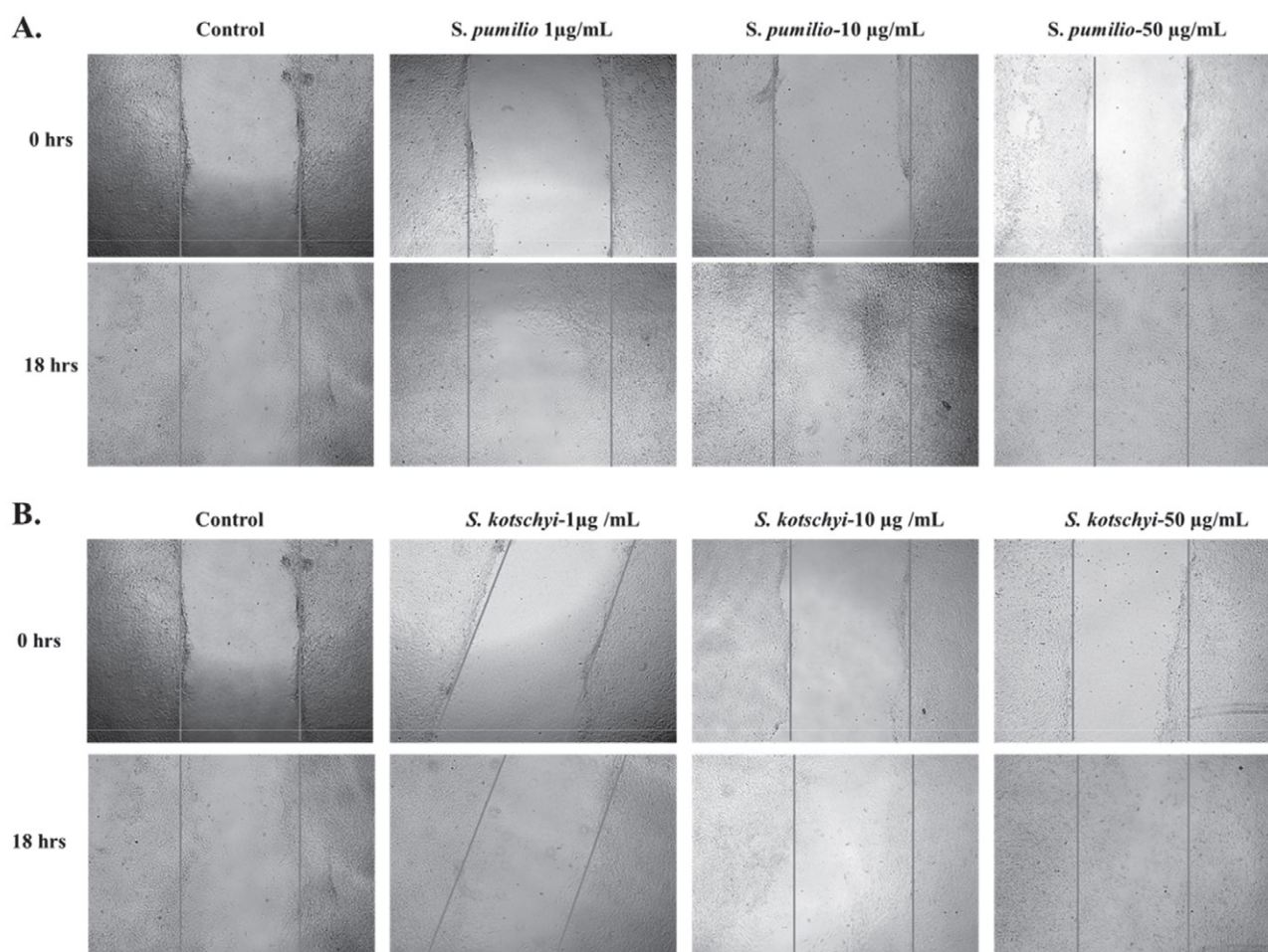


Figure 3. The results of wound healing assay for *S. kotschyi* and *S. pumilio* extracts.

for *S. kotschyi* and 58.03% for *S. pumilio* by the 18th hour of the experiment.

Table 5. Percentage of wound closure induced by plant extracts in NIH-3T3 fibroblast cells (mean ± SD).

	Wound Closure (%)	
	<i>S. kotschyi</i>	<i>S. pumilio</i>
Control	56.75 ± 2.04 ^a	58.03 ± 2.61 ^a
1 µg/mL	38.71 ± 1.34 ^a	68.25 ± 3.74 ^b
10 µg/mL	79.43 ± 3.02 ^a	87.33 ± 2.73 ^b
50 µg/mL	92.61 ± 3.51 ^a	100.00 ± 3.21 ^a

^{a,b} Data marked with different letters in the same row indicate significant differences ($p < 0.05$).

In the same examination, it was observed that *S. pumilio* extract at concentrations of 1, 10, and 50 µg/mL significantly promoted wound closure in fibroblast cells, with healing rates of 68.25%, 87.33%, and 100.00%, respectively, compared to the control group ($p < 0.05$). Conversely, the *S. kotschyi* extract did not show a significant effect on wound healing at the lowest concentration (1 µg/mL), as it was comparable to the control ($p > 0.05$). However, at 10 and 50 µg/mL, the *S. kotschyi* extract demonstrated a significant improvement in wound closure (Table 5) ($p < 0.05$). Furthermore, when comparing the two species, *S. pumilio* extracts were found to support wound healing significantly more than *S. kotschyi* across all tested concentrations, indicating a superior efficacy in promoting cell regeneration in fibroblast cells.

Quantitatively, the higher concentration of caffeic acid in *S. pumilio* (10,564.70 µg/g) compared to *S. kotschyi* (3,377.46 µg/g) may explain its superior efficacy in wound healing. Caffeic acid, with its potent antioxidant and anti-inflammatory properties, along with its derivatives, has been recognized as a promising alternative to current pharmacological treatments for various skin injuries and incisional wounds. By promoting healing processes, it plays a critical role in tissue repair and regeneration.⁴⁰

The results indicate that *S. kotschyi* and *S. pumilio* extracts hold promise for applications in cancer therapy, particularly for cervical cancer and neuroblastoma, as well as in tissue regeneration. However, their clinical translation requires comprehensive in vivo studies to evaluate their pharmacokinetics, bioavailability, and long-term effects. Additionally, the development of formulations to enhance their stability and reduce toxicity will be critical for integrating these extracts into pharmaceutical practice.

The results of this study highlight the significant pharmacological potential of *S. kotschyi* and *S. pumilio*, but certain limitations must be addressed to contextualize these findings fully. While the extracts showed promising cytotoxic effects against cancer cell lines and wound heal-

ing capabilities, the observed toxicity of *S. kotschyi* towards healthy fibroblast cells underlines the need for strategies to improve selectivity and safety. In addition, the in vitro nature of the study, the limited number of cell lines tested and the partial characterization of the phenolic compounds represent areas for further investigation. Comprehensive in vivo studies are needed to assess pharmacokinetics, bioavailability and long-term effects, while identification of specific bioactive compounds could elucidate the mechanisms underlying the observed effects. Despite these limitations, the observed interspecies differences, such as the superior wound healing efficacy and lower toxicity of *S. pumilio*, highlight its potential as a safer and more effective candidate for therapeutic applications. These findings not only contribute to the understanding of the bioactivity of underexplored plant species but also pave the way for their future development in pharmaceutical practice.

In addition, the mechanisms underlying the observed cytotoxicity and tissue regenerative effects should be further investigated. Phenolic compounds, especially caffeic acid, may exert these effects through antioxidant, anti-inflammatory and cell signalling pathways, which could be elucidated by molecular and pathway-based studies. In the future, refinement of extracts and advanced studies, including in animal models, will be essential to bridge the gap between in vitro findings and potential clinical applications to ensure safe and effective therapeutic use of these underexplored species.

4. Conclusion

In conclusion, this study represents the first comprehensive investigation into the phytochemical composition of the endemic *Saponaria kotschyi* and *Saponaria pumilio* extracts, highlighting their remarkable richness in secondary metabolites and substantial antioxidant activity. The findings emphasize the diverse phenolic profiles of these species, showcasing their potential for therapeutic applications in cervical cancer, neuroblastoma, and tissue repair and regeneration. This work fills an important gap in understanding *Saponaria*'s phenolic compounds. However, the study's in vitro nature, limited number of tested cell lines, and observed toxicity toward healthy cells underscore the need for in vivo studies, deeper mechanistic insights, and strategies to improve selectivity. Addressing these limitations and further exploring interspecies differences will provide a stronger foundation for future clinical applications.

Acknowledgments

This study was financially supported by the Scientific Research Projects Coordination Unit (Project no: 2018FEBE043), Pamukkale University, Türkiye.

5. References

1. J. Noroozi, G. Zare, M. Sherafati, M. Mahmoodi, D. Moser, Z. Asgarpour, G. M. Schneewiss, *Front. Ecol. Evol.* **2019**, *7*, 159. DOI:10.3389/fevo.2019.00159
2. M. Ekor, *Front Pharmacol.* **2014**, *4*, 177. DOI:10.3389/fphar.2013.00177
3. C. Ozay, *Riv Ital Sostanze Gr.* **2023**, *100*, 177–185.
4. M. Erdir, E. Ataşlar, *Phytotaxa* **2022**, *566*, 73–88. DOI:10.11646/phytotaxa.566.1.4
5. G. M. Petrović, M. D. Ilić, V. P. Stankov-Jovanović, G. S. Stojanović, S. Č. Jovanović, *Nat Prod Res.* **2018**, *32*, 331–334. DOI:10.1080/14786419.2017.1350668
6. J. P. Vincken, L. Heng, A. de Grot, H. Gruppen, *Phytochem.* **2007**, *68*, 275–297. DOI:10.1016/j.phytochem.2006.10.008
7. Y. Lu, D. Van, L. Deibert, G. Bishop, J. Balsevich, *Phytochem.* **2015**, *113*, 108–120. DOI:10.1016/j.phytochem.2014.11.021
8. D. Charalambous, M. Christoforou, E. N. Kitiri, M. Andreou, D. Partassides, C. Papachrysostomou, M. Frantzi, G. A. Karikas, M. Pantelidou, *Molecules* **2022**, *27*, 5812. DOI:10.3390/molecules27185812
9. M. Sengul, S. Ercisli, H. Yildiz, N. Gungor, A. Kavaz, B. Çetin, *Iran J Pharm Res.* **2011**, *10*, 49–56. PMID:24363680
10. H. Azaizah, B. Saad, K. Khalil, O. Said, *Evid Based Comp Alternat Med.* **2006**, *3*, 229–235. DOI:10.1093/ecam/nel034
11. V. López, S. Akerreta, E. Casanova, J. M. García-Mina, R. Y. Caverro, M. I. Calvo, *Plant Food Hum Nutr.* **2007**, *62*, 151–155. DOI:10.1007/s11130-007-0056-6
12. W. Sun, M. H. Shahrajabian, *Molecules* **2023**, *28*, 1845. DOI:10.3390/molecules28041845
13. A. Grzywaczyk, W. Smulek, E. Kaczorek, *World J Microbiol Biotechnol.* **2024**, *40*. DOI:10.1007/s11274-024-03961-9
14. C. Ozay, R. Mammadov, *Acta Biol. Hung.* **2017**, *68*, 310–320. DOI:10.1556/018.68.2017.3.8
15. R. Mammadov, A. Kaska, C. Ozay, *Indian J Pharm Sci.* **2017**, *79*, 585–590. DOI:10.4172/pharmaceutical-sciences.1000266
16. J. Bekir, M. Mars, J. P. Souchard, J. Bouajila, *Food Chem Toxicol.* **2013**, *55*, 470–475. DOI:10.1016/j.fct.2013.01.036
17. R. Re, N. Pellegrini, A. Proteggente, A. Pannala, M. Yang, C. Rice-Evans, *Free Radic Biol Med.* **1999**, *26*, 1231–1237. DOI:10.1016/S0891-5849(98)00315-3
18. R. Apak, M. Özyürek, K. Güçlü, E. Çapanoğlu, *J Agric Food Chem.* **2016**, *64*, 997–1027. DOI:10.1021/acs.jafc.5b04739
19. P. Prieto, M. Pineda, M. Aguilar, *Anal Biochem.* **1999**, *269*, 337–341. DOI:10.1006/abio.1999.4019
20. T. C. Dinis, V. M. Maderia, L. M. Almeida, *Arch Biochem Biophys.* **1994**, *315*, 161–169. DOI:10.1006/abbi.1994.1485
21. F. Aydın Köse, İ. Öztürk, S. Cin, Ş. Baykan, *Eur J Ther.* **2022**, *28*, 45–51. DOI:10.54614/eurjther.2022.0113
22. G. Cory, *Methods Mol Biol.* **2011**, *769*, 25–30. DOI:10.1007/978-1-61779-207-6_2
23. Q. D. Do, A. E. Angkawijaya, P. L. Tran-Nguyen, L. H. Huynh, F. E. Soetaredjo, S. Ismadji, Y. H. Ju, *J Food Drug Anal.* **2014**, *22*, 296–302. DOI:10.1016/j.jfda.2013.11.001
24. R. Verpoorte, *J Pharm Pharmacol.* **2000**, *52*, 253–262. DOI:10.1211/0022357001773931
25. S. Chandra, D. S. Rawat, A. Bhatt, *Not Sci Biol.* **2021**, *13*, 10809–10809. DOI:10.15835/nsb13110809
26. D. Charalambous, M. Christoforou, K. Christou, M. Christou, A. Ververis, M. Andreou, K. Christodoulou, A. Koutsoulidou, C. Papachrysostomou, M. Pantelidou, *Plants* **2024**, *13*, 1982. DOI:10.3390/plants13141982
27. W. Smulek, A. Zdarta, A. Pacholak, A. Zgoła-Grześkowiak, Ł. Marczak, M. Jarzębski, E. Kaczorek, *Colloids and Surf B: Biointerfaces* **2017**, *150*, 209–215. DOI:10.1016/j.colsurfb.2016.11.035
28. C. Özay, E. Pehlivan, *Ankara Ecz Fak Derg.* **2024**, *8*, 1248–1263. DOI:10.33483/jfpau.1488042
29. E. Bursal, A. Aras, M. Doğru, Ö. Kılıç, *Int J Life Sci Biotech.* **2022**, *5*, 1–8. DOI:10.38001/ijlsb.989172
30. G. A. Agbor, J. R. Kuiafe, E. Sangiovanni, O. O. Ojo, Volume II. *Front Pharmacol* **2023**, *14*, 1310291. DOI:10.3389/fphar.2022.957296
31. N. V. Nguyen, N. T. Duong, K. H. Nguyen, N. T. Bui, T. Thi Pham, K. T. Nguyen, P. H. Lee, K. H. Kim, *Biointerface Res Appl Chem.* **2022**, *12*, 2678–2690. DOI:10.33263/BRIAC122.26782690
32. P. Neupane, J. Lamichhane, *Vegetos* **2020**, *33*, 360–366. DOI:10.1007/s42535-020-00116-7
33. S. Waghulde, M. K. Kale, V. Patil, *Proceedings* **2019**, *41*, 47. DOI:10.3390/ecsoc-23-06703
34. M. Gulfishan, M. Afzal, I. Kazmi, A. M. Quazi, T. A. Bhat, A. Jahan, Springer, **2018**, pp. 337–360. DOI:10.1007/978-981-10-8417-1_14
35. J. J. Balsevich, I. Ramirez-Erosa, R. A. Hickie, D. M. Dunlop, G. G. Bishop, L. K. Deibert, *Fitoterapia* **2012**, *83*, 170–181. DOI:10.1016/j.fitote.2011.10.010
36. M. K. Erdogan, I. H. Gecibesler, Y. Yapar, R. Gundogdu, M. Kirici, L. Behcet, P. Taslimi, *Bioorg Chem* **2021**, *113*, 105032. DOI:10.1016/j.bioorg.2021.105032
37. S. R. Kotian, K. S. R. Pai, J. K. Nayak, H. Bangera, K. Prasad, K. M. R. Bhat, *Int J Pharm Pharm Sci.* **2015**, *7*, 163–171.
38. O. A. Hanafiah, T. Abidin, S. Ilyas, M. Nainggolan, E. Syamsudin, *J Int Dent Med Res.* **2019**, *12*, 854–858.
39. N. Shah, S. Mitra, U. Sharma, K. Sharma, *Int J Ayurveda Pharma Res.* **2022**, *10*, 13–21. DOI:10.47070/ijapr.v10i4.2274
40. N. A. Abdul Jalil, F. Ahmad, D. Sankar, M. F. Hasnol, N. A. Azahari, S. A. Syed Shahrin, Z. Wong, *Med and Health* **2024**, *19*, 25–39. DOI:10.17576/MH.2024.1901.02

Povzetek

Raziskali smo skupno količino sekundarnih metabolitov, fenolni profil, antioksidativno aktivnost, akutno toksičnost, citotoksične učinke in učinke na celjenje ran iz izvlečkov nadzemnih delov *Saponaria kotschy* (endemit) in *Saponaria pumilio*. Ovrednotili smo skupno vsebnost fenolov, flavonoidov, saponinov in taninov; antioksidativno aktivnost (s šesti-
mi različnimi metodami, vključno s testi β -karotena/linolenske kisline, DPPH, ABTS, FRAP, fosfomolibdena in keliranja kovin); fenolne sestavine v ekstraktih (metoda HPLC); akutno toksičnost (test smrtnosti slanih kozic); antiproliferativne učinke na celične linije človeškega raka materničnega vratu (HeLa) in človeškega nevroblastoma (SH-SY5Y) (test MTT); in učinke na celjenje ran v zdravih mišjih fibroblastnih celicah (NIH-3T3) z in vitro testom praskanja. Metanolni izvleček *S. kotschy* je pokazal višje koncentracije sekundarnih metabolitov in antioksidativno aktivnost kot izvleček *S. pumilio*. Obe vrsti sta vsebovali predvsem kavno kislino, 2,5-dihidroksibenzojsko kislino in epikatehin, pri čemer je bila vsebnost kavne kisline v *S. pumilio* izrazito višja. Poleg tega je izvleček *S. kotschy* izkazoval večjo citotoksičnost proti rakavim celicam HeLa in SH-SY5Y, medtem ko je *S. pumilio* izkazoval večjo učinkovitost pri celjenju ran. Dve prej neraziskani vrsti *Saponaria* razkrivata izjemno bogastvo sekundarnih metabolitov in močno antioksidativno aktivnost. Zaradi različnih fenolnih sestavin se te vrste kažejo kot obetavni kandidati za terapevtsko uporabo pri raku materničnega vratu in nevroblastomu ter imajo potencialno ključno vlogo pri obnovi in regeneraciji tkiv – področja, ki si zaslužijo nadaljnje poglobljene raziskave.



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