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# ANALYSIS OF THE PHYTOCHEMICAL COMPOSITION OF *SAPONARIA OFFICINALIS* L. SPECIES IN THE FLORA OF THE NAKHCHIVAN AUTONOMOUS REPUBLIC

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**Abstract**

*The growing demand for safe, natural alternatives to synthetic antioxidants—many of which are linked to adverse health effects—has intensified interest in plant-derived bioactive compounds. This study investigates the phytochemical composition and antioxidant activity of Saponaria officinalis L. (Caryophyllaceae), cultivated within the cultural flora of the Nakhchivan Autonomous Republic. Chromatographic and spectrophotometric techniques were employed to identify key classes of secondary metabolites, including flavonoids, carotenoids, anthocyanins, alkaloids, and saponins.*

*Ethanol extracts of leaves and flowers demonstrated strong antioxidant potential, with DPPH radical-scavenging inhibition rates of 87.6% and 86.4% at 50 mg/mL concentration, respectively. Chromatographic analysis revealed the presence of flavone, 3-hydroxyflavone, and β-carotene in leaf extracts, as well as apigenin, flavanones, and hydroxyflavones in flower extracts. Spectrophotometric data identified chlorophyll peaks at 664 nm and carotenoid absorption at 470 nm. The total phenolic content of aerial parts was quantified as 7.12 µg/mg gallic acid equivalents (GAE).*

*These findings suggest that S. officinalis L. is a potent natural source of antioxidant compounds with potential applications in pharmaceutical, nutraceutical, and cosmetic industries as an alternative to synthetic antioxidants.*

***Keywords:*** *Saponaria officinalis L.; antioxidant activity; phytochemicals; flavonoids; DPPH assay; phenolic content; chromatography; spectrophotometry.*

## INTRODUCTION

In recent years, scientific and regulatory concerns over the safety of synthetic antioxidants—such as butylated hydroxytoluene (BHT) and butylated hydroxyanisole (BHA)—have driven increased interest in natural alternatives. Oxidative stress, resulting from an imbalance between reactive oxygen species (ROS) and the body's antioxidant defense mechanisms, plays a critical role in the pathogenesis of numerous degenerative conditions, including cardiovascular diseases, cancer, and neurodegenerative disorders. One of the primary manifestations of oxidative stress is lipid peroxidation, wherein ROS attack polyunsaturated fatty acids, leading to membrane damage and the formation of harmful metabolites.

As a result, research into plant-derived antioxidants capable of neutralizing free radicals has intensified. Among promising candidates is *Saponaria officinalis* L., commonly known as soapwort, a perennial species from the Caryophyllaceae family. This plant, cultivated within the cultural flora of the Nakhchivan Autonomous Republic, has historically been used in folk medicine as a cleanser, diuretic, and expectorant. Recent studies (Guliyev & Mansur, 1999; Dönmez & Yılmaz, 2024; Ekşi Bona, 2025) confirm its rich phytochemical profile, including saponins, flavonoids, phenolic acids, carotenoids, and alkaloids—compounds widely recognized for their antioxidant properties.

The present study provides a detailed analysis of the phytochemical composition and antioxidant capacity of *S. officinalis* L., using chromatographic and spectrophotometric techniques. Specific compounds such as flavone, 3-hydroxyflavone, β-carotene, and apigenin were identified, while antioxidant activity was assessed using DPPH free radical scavenging assays and colorimetric reactions. Notably, phenolic content and flavonoid derivatives were found to contribute significantly to antioxidant behavior.

This research contributes to the growing body of evidence supporting the pharmaceutical and nutraceutical relevance of *S. officinalis* L. and highlights its potential role as a natural antioxidant source for industrial applications.

## MATERIALS AND METHODS

The species *Saponaria officinalis* L., selected as the object of the study, was prepared for analysis at the *Biochemical Research Laboratory* of Nakhchivan State University. Plant samples were collected and dried to a constant weight, after which their mass was measured.

In the study of the phytochemical composition of the species, modern analytical methods such as chromatography and spectrophotometry were primarily used. Chromatography is one of the most effective and universal physicochemical techniques for separating and analyzing complex mixtures of substances. Today, the chromatographic method is widely applied in various research fields, particularly in the analysis of food product composition and the assessment of their quality parameters (Alpınar, 2015; Mete, 2009; Wu, Raven, & Hong, 2001).

The main purpose of using spectroscopic analysis methods was to identify plant-derived compounds such as flavonoids, carotenoids, anthocyanins, alkaloids, and other classes of bioactive substances.

Spectral analyses were performed using a *Hitachi U-2900 UV-VIS spectrophotometer*, while chromatographic analyses were carried out using a *60108-712 HYPERSEP SI column* (10 g/75 mL/10 PKG) (Figure 1) and *DC-fertigfolien ALUGRAM SIL G/UV254* thin-layer plates. To determine the composition of the extracts, both column and thin-layer chromatography were employed using the following solvent systems:

- Acetic acid + n-butanol + water (1:4:5)
- Petroleum ether + acetone + water (3:1:1) (Mabry, Markham, & Thomson, 1970)

Thin-layer chromatography was performed on standard silica gel plates (Kieselgel G60 F254, Merck). Using a micropipette, 30 µL of the test extract solution and, in parallel, 10 µL of 1% solutions of standard compounds (rutin, quercetin, isoquercitrin, etc.) were applied as spots along the starting line of the plates.

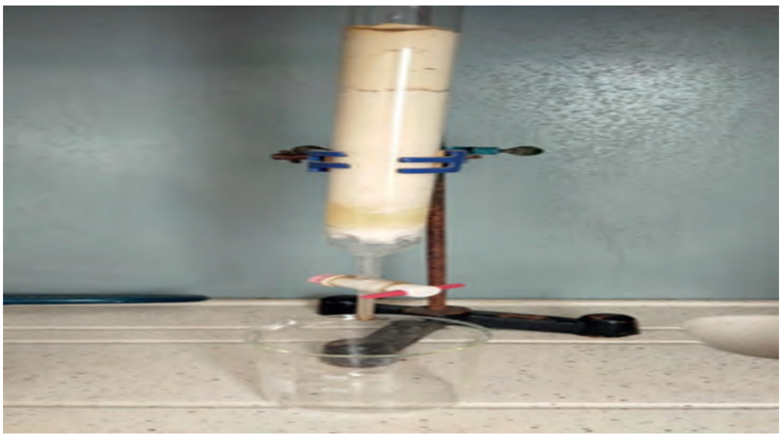

**Figure 1.** Column chromatography

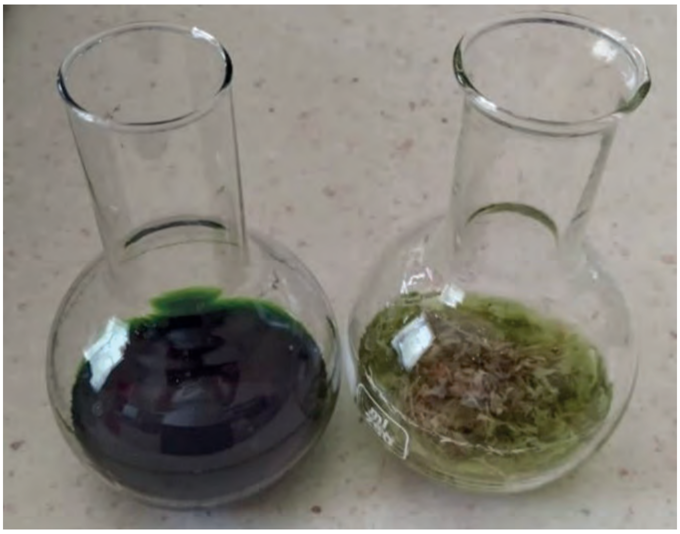

**Figure 2.** Extraction of Stems and Flowers of Saponaria officinalis L. (1. Leaf extract; 2. Flower extract)

During the chromatographic procedure, the plates were first placed in chambers pre-saturated with eluent vapors. After the solvent front had migrated 11 cm, the plates were removed from the chamber and air-dried until the solvents had completely evaporated. The resulting chromatograms were examined under UV light (Model CM-10A fluorescence analysis cabinet) at wavelengths of 254 nm and 365 nm. The positions and colors of the spots were recorded (Prior, Wu, & Scaich, 2005).

The leaves, flowers, and stems of *Saponaria officinalis* L. were dried separately according to standard methods and ground using a blender. The dried plant materials were extracted with various solvents for 3 hours, in two repetitions, and the resulting extracts were filtered.

The flowers were first extracted with hexane and ethanol, followed by extraction with 0.5% hydrochloric acid in ethanol. The leaves were extracted using hexane and ethanol, while an aqueous extract was prepared from the stems. The extracts were concentrated under vacuum using a rotary evaporator (Figure 2).

The absorbance spectra of the obtained extracts were measured with a UV spectrophotometer, and based on the wavelength values, the compounds present in the extracts were identified.

## METHODS FOR THE STUDY OF FLAVONOIDS

The qualitative determination of flavonoids in extracts obtained from different plant organs was carried out using two characteristic reactions: the ferric (III) chloride reaction and the cyanidin reaction. The interaction of flavonoids with ferric (III) chloride produces green-colored complexes for flavonols (rutin, quercetin, kaempferol, etc.) and brown-colored complexes for flavanones.

In the cyanidin reaction, flavonols and flavones are reduced in the presence of magnesium powder and HCl, resulting in the formation of anthocyanidins that produce red or orange coloration (Velioglu, Mazza, Gao, & Omah, 1998).

Chalcones and aurones do not give a reaction in the cyanidin test; however, when concentrated HCl is added to their solutions (without magnesium), red coloration appears due to the formation of oxonium salts. To 2 mL of the extract, 5–7 drops of concentrated HCl and 10–15 mg of magnesium powder are added. After 3–5 minutes, a gradual color change is observed. To accelerate the reaction and enhance color intensity, the reaction tube can be heated in a boiling water bath for 2–3 minutes (Rahimova & Novruzova, 2023; Johnson, Foster, Low, & Kiefer, 2017; Yenigün & Başar, 2024).

## METHODS FOR THE STUDY OF SAPONINS

The presence of saponins in extracts was determined by several qualitative tests. The extract was dissolved in 20 mL of distilled water and stirred for 15 minutes. The formation of a 1 cm foam layer indicated the presence of saponins. To the aqueous saponin extract, 1 mL of ethanol, 1 mL of concentrated $H_2SO_4$, and 1 drop of 10% $FeSO_4$ solution were added. Upon heating, a blue-green color developed (Lafon reaction).

## METHODS FOR THE STUDY OF PHENOLIC COMPOUNDS

The total phenolic content of the plant extract was determined using the Folin–Ciocalteu method. For quantification, 0.5 mL of Folin–Ciocalteu reagent (1 N), 7.5 mL of water, 1.5 mL of 10% $Na_2CO_3$, and 1 mL of the extract were mixed. The mixture was allowed to stand for 90 minutes, and the absorbance was measured at 765 nm. Gallic acid (1 mL) was used as the standard.

## RESULTS AND DISCUSSION

Information regarding the qualitative composition and quantitative characteristics of *Saponaria officinalis* L. distributed within the territory of the Nakhchivan Autonomous Republic is limited. Therefore, the present study was undertaken to obtain comprehensive data on the qualitative composition and quantitative indicators of various organs of this species, as well as its overall phytochemical profile.

The leaves, flowers, and stems of the plant were extracted with various solvents, after which the extracts were filtered, concentrated using a rotary evaporator, and analyzed spectrophotometrically. The absorbance spectra were recorded using a UV spectrophotometer, while chromatographic analyses were performed to identify the compounds present in the extracts based on their *Rf* values.

Chromatographic data revealed the presence of flavone, 3-hydroxyflavone, and β-carotene in the leaf extracts of the plant. In the acidified ethanol extract of the flowers, apigenin, flavone, two hydroxyflavone-type compounds (3-hydroxyflavone and 6-hydroxyflavone), as well as flavanone compounds were identified. These findings demonstrate that *Saponaria officinalis* L. possesses a diverse range of flavonoid derivatives, indicating its potential antioxidant and pharmacological activity, consistent with previous research on the Caryophyllaceae family (Velioglu et al., 1998; Prior et al., 2005; Rahimova & Novruzova, 2023). Table 1 presents the chromatographic analysis results of *Saponaria officinalis* L. extracts, showing the *Rf* values obtained for individual compounds.

**Table 1. Chromatographic analysis results of *Saponaria officinalis* L. extracts**

| Extract Type | Identified Compounds | Rf Values |
|---|---|---|
| Leaf Extract | Flavone | 0.91 |
| | 3-Hydroxyflavone | 0.56 |
| | β-Carotene | 0.97 |
| Flower Extract | Apigenin | 0.21 |
| | 6-Hydroxyflavone | 0.46 |
| | 3-Hydroxyflavone | 0.56 |
| | Flavanone | 0.75 |

The pigments present in the leaf extracts of *Saponaria officinalis* L. were analyzed using both chromatographic and spectrophotometric techniques. A prominent absorption peak was recorded at **664 nm**, corresponding to **chlorophyll *a***, thereby confirming its presence in the leaf extract ( *Figure 3*). This result aligns with the characteristic absorption maxima of chlorophyll *a* in higher plants, as reported in previous studies (Mabry, Markham, & Thomson, 1970; Johnson, Foster, Low, & Kiefer, 2017).

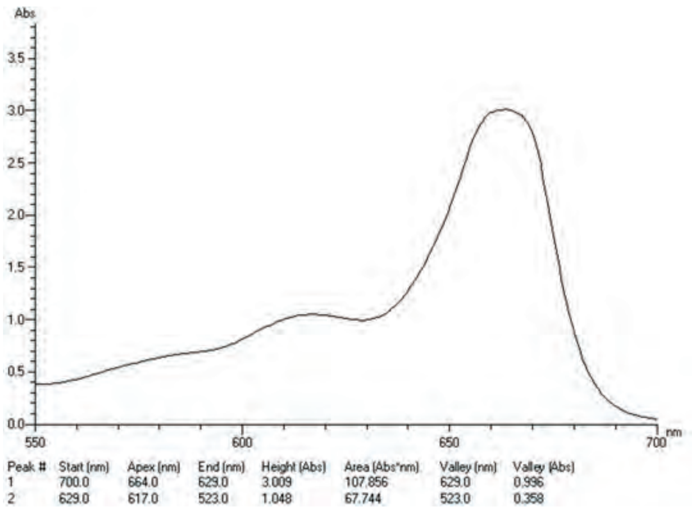


**Figure 3.** UV spectrum of chlorophyll A pigment in the leaf extract of *Saponaria officinalis L.*

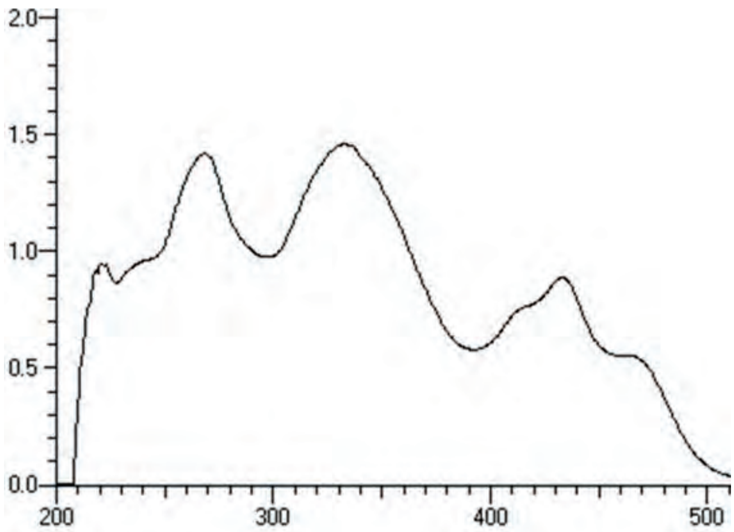


**Figure 4.** UV spectrum of the ethanol extract of the leaves of Saponaria officinalis L.

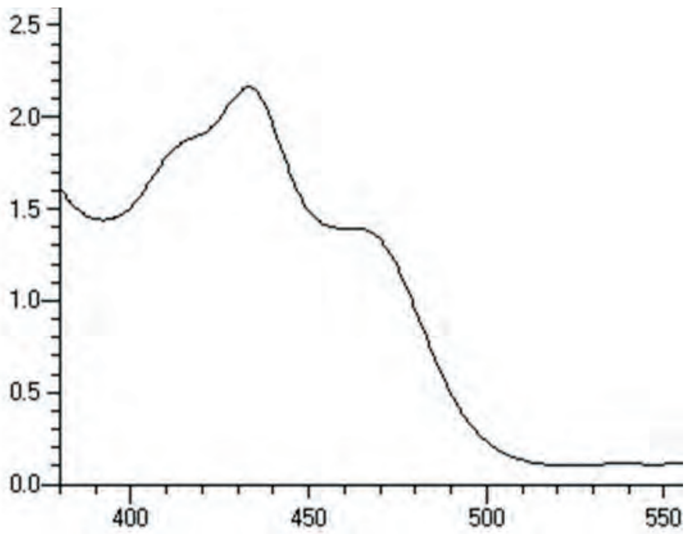


**Figure 5.** UV spectrum of Saponaria officinalis L. flower extract

Based on the wavelengths obtained with a UV spectrophotometer from the ethanol extract of the plant leaves, the compounds present in the extract were identified, as shown in Figure 4.

The observed wavelengths of 268 nm and 333 nm correspond to flavones, specifically apigenin 7-O-glucoside. Additionally, a wavelength of 280 nm was recorded, which is characteristic of phenolic compounds.

In the flowers of the plant, peaks at a wavelength of 470 nm, corresponding to carotenoid compounds, were recorded, as shown in Figure 4.

*Saponaria officinalis* L. is rich in flavonoids and phenolic compounds. The total amount of phenolic compounds in the aerial parts of the plant was determined, and the total phenolic content was found to be 7.12 µg/mg in gallic acid equivalents.

The qualitative composition of saponins in the stems of the plant was determined using the Lafon reaction. The formation of a 1 cm foam layer and the appearance of a blue-green color upon heating indicate the presence of saponins in the extract [Güçlü-Üstündağ, Ö.; Mazza, G., 2007; Mehra, N., 2023].

The free radical scavenging activities of the extracts were determined using the 2,2-diphenyl-1-picrylhydrazyl (DPPH) method. During the reaction, the decrease in the amount of DPPH radicals was measured by recording the absorbance of the reaction mixture at a wavelength of 517 nm. A decrease in absorbance indicates a reduction in the amount of DPPH in the reaction medium. The DPPH free radical scavenging activity of the ethanol extract of the leaves of Saponaria officinalis was 87.6% at a concentration of 50 mg/ml, while the DPPH free radical scavenging activity of the flower extract was 86.4% at the same concentration. At the same time, a strong correlation between the total phenolic content and antioxidant activity in the plant was observed (Figure 6).

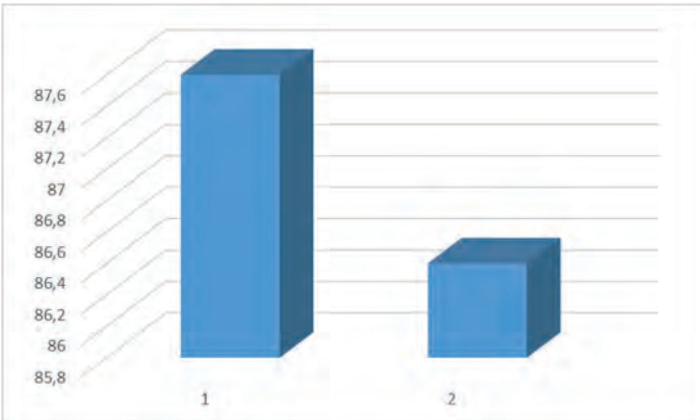


**Figure 6.** DPPH free radical scavenging activity of leaf and flower extracts of Saponaria officinalis
1. *Free Radical Scavenging Activity of the Leaf Extract*
2. *Free Radical Scavenging Activity of the Flower Extract*

## CONCLUSION

*Saponaria officinalis* L., cultivated in the cultural flora of the Nakhchivan Autonomous Republic, was comprehensively studied under laboratory conditions to determine its phytochemical composition and antioxidant potential. During the study, leaves, flowers, and stems of the plant were extracted using various solvents. The resulting extracts were filtered, concentrated with a rotary evaporator, and subjected to thin-layer and column chromatography. Their absorbance spectra were measured using UV–visible spectrophotometry. Based on Rf values obtained from chromatographic analyses, several phytochemical compounds were identified.

In the leaf extracts, flavone, 3-hydroxyflavone, and β-carotene were detected. In the acidified ethanol extract of the flowers, apigenin, flavone, and two hydroxyflavone-type compounds—3-hydroxyflavone and 6-hydroxyflavone—along with flavanone compounds were identified. The aqueous extract of the stems tested positive for saponins, confirming the presence of triterpenoid glycosides. The total phenolic content in the aerial parts of the plant was quantified as 7.12 µg/mg in gallic acid equivalents (GAE), indicating a significant presence of polyphenolic antioxidants.

The antioxidant activity of the leaves and flowers was assessed using classical qualitative reactions. In the ferric (III) chloride test, the formation of green-colored complexes for flavonols (such as rutin, quercetin, and kaempferol) and brown-colored complexes for flavanones confirmed the presence of structurally diverse flavonoids. In the cyanidin reaction, the reduction of flavones in the presence of magnesium powder and HCl produced red or orange-colored anthocyanidins, further demonstrating the presence of flavonoid derivatives.

Quantitative evaluation of antioxidant capacity was conducted using the DPPH free radical scavenging assay. The ethanol extract of the leaves exhibited 87.6% inhibition at a concentration of 50 mg/mL, while the flower extract showed 86.4% inhibition at the same concentration. These values confirm the strong antioxidant activity of *S. officinalis* L. extracts.

Spectrophotometric analysis revealed a chlorophyll *a* absorption peak at 664 nm in the leaf extract, and characteristic carotenoid peaks at 470 nm in the flower extract. These findings support the presence of photosynthetic pigments alongside bioactive flavonoids and phenolics.

In summary, *Saponaria officinalis* L. demonstrates a rich phytochemical profile, including flavonoids, phenolics, carotenoids, saponins, and chlorophyll pigments, with substantial antioxidant potential. The high levels of radical scavenging activity and phenolic content suggest promising applications in the development of natural antioxidant agents for use in pharmaceutical, nutraceutical, and cosmetic industries. Further research is warranted to isolate individual bioactive compounds and evaluate their specific mechanisms of action in biological systems.

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