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RESEARCH ARTICLE

# Phytochemical Composition and Multifunctional Bioactivities of *Salvia syriaca* L.: A Promising Natural Source for Antioxidant and Pest Management Applications

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## ABSTRACT

This study evaluated the phenolic composition, antioxidant capacity, and biological activities of the aerial parts (leaves, shoots, and flowers) of *Salvia syriaca* L. (Syrian sage). Samples were collected at the flowering stage during the 2024 vegetation period. The methanolic extract was analyzed by LC-MS/MS, identifying nine phenolic compounds. Ferulic acid (2850.43 ± 65.55 mg/kg DW (Dry Weight), rutin (2014.45 ± 3.97 mg/kg DW), and rosmarinic acid (82.50 ± 3.52 mg/kg DW) were the predominant constituents. Total phenolic content (TPC) was 37.42, 34.07, and 14.40 mg GAE/g extract in ethyl acetate, methanol, and hexane extracts, respectively, while total flavonoid content (TFC) was 23.29, 21.71, and 7.91 mg QE/g extract. Methanol and ethyl acetate showed stronger DPPH radical scavenging activity than hexane. The ethyl acetate extract exhibited the highest FRAP value (1.13 µmol TE/mg extract), while methanol showed superior CUPRAC activity (6.89 µmol TE/mg extract). Biological assays indicated that the methanolic extract inhibited germination of weeds, growth of plant pathogenic fungi, and bacterial pathogens and caused high mortality in the stored grain pest *Sitophilus granarius*. These findings suggest that *S. syriaca* is a promising natural source of bioactive compounds for integrated pest management (IPM).

## 1 | Introduction

Allelopathy is derived from the Greek words *allelo* and *pathos*, meaning 'one harming another.' In other words, it refers to the positive or negative effects a plant exerts on other plants through the secondary metabolites it produces and releases (Rice 1984; Rizvi et al. 1992). The concept of allelopathy, which is associated with secondary compounds involved in plant-to-plant interactions in nature, has now been incorporated into the studies of many pharmacologists, herbologists, ecologists, and physiologists. To understand the concept and mechanism of allelopathy, it is crucial to comprehend the effects and causes of allelochemicals on plants. These secondary metabolites, referred to as allelochemicals, have been reported to affect germination, shoot and root elongation (Li et al. 2020; Cheng et al. 2024), cell division, ion and water uptake, phytohormone metabolism, respiration, photosynthesis, enzyme activities, and even gene expression (Singh and Thapar 2003).

These plant-produced allelochemicals not only contribute to allelopathic interactions but can also negatively impact plant pathogenic fungi, bacterial diseases, and insects (Erez and Battal 2022; Dayan and Duke 2009). For this reason, studies on the use of these natural compounds in the control of pests and diseases have been conducted and are still ongoing. Considering the negative effects of synthetic pesticides heavily used in agriculture on human health and the environment, research into such natural compounds is of critical importance. In the control of plant pathogenic bacteria, cultural practices are often insufficient, and effective chemical agents are lacking. The use of agricultural antibiotics is also prohibited. Therefore, the development of

**Abbreviations:** DW, dry weight; DPPH, 2,2-diphenyl-1-picrylhydrazyl; FRAP, ferric reducing antioxidant power; MIC, minimum inhibitory concentration; TFC, total flavonoid contents; TPC, total phenolic contents.

alternative methods and new agents is essential. In recent years, there has been a notable increase in research on medicinal and aromatic plants, which can be considered natural antibiotics, and the antimicrobial compounds derived from them.

Available phytochemical studies on *S. syriaca* have mainly focused on the aerial parts and demonstrate that this species possesses a relatively complex and phenolic-rich profile. Methanolic extracts of the above-ground organs exhibit high total phenolic and flavonoid contents and are particularly enriched in hydroxycinnamic acids (e.g., rosmarinic, caffeic, and ferulic acids) and flavonoids such as rutin, quercetin, and apigenin, together with several simpler phenolic acids (Kurşat and Emre 2019).

The cytotoxic (Arslan et al. 2021), antioxidant (Bahadori et al. 2017), and antimicrobial (Karataş and Ertekin 2010; Firuzi et al. 2013) effects of extracts from *Salvia syriaca*, obtained using different solvents, have been demonstrated in various studies. For instance, in a study using methanol extracts of *S. syriaca* collected in Iran, antibacterial activity was reported against both Gram-positive and Gram-negative bacteria (*Escherichia coli*, *Klebsiella pneumoniae*, *Salmonella typhi*, *Staphylococcus aureus*, *S. epidermidis*, *Bacillus subtilis*) (Firuzi et al. 2013). Another study examining various *Salvia* species, including *S. syriaca*, found that a dose of 30 mg/mL had antimicrobial effects against *Bacillus subtilis*, *Staphylococcus aureus*, and *Staphylococcus epidermidis* (Iravani et al. 2019).

Like bacteria, plant pathogenic fungi are major threats in crop production, causing significant yield losses and typically controlled through fungicides. However, the excessive use of fungicides has raised concerns due to their negative impacts on human health and the environment and the development of resistance in pathogens (Bastos et al. 2021). *Sitophilus granarius* L. (*Coleoptera: Curculionidae*) is an important pest of stored wheat (Schwartz and Burkholder 1991), and both the larvae and adults are capable of causing damage (Fava and Burlando 1995). Although fumigants are often used for control, their toxicity and application difficulties have driven the search for alternative strategies. In recent years, research on alternative control methods has increased significantly, particularly focused on the use of secondary metabolites found in plant-based pesticides as promising agents against weeds, plant pathogenic bacteria, fungi, and insect pests, which are major contributors to yield losses in agriculture.

This study was conducted to investigate the chemical profile (HPLC), antioxidant activity, as well as allelopathic, antifungal, antibacterial, and insecticidal activities of *Salvia syriaca* L., a species naturally distributed within the flora of Turkey.

## 2 | Material and Methods

### 2.1 | Plant Material

*Salvia syriaca* is a plant species that naturally occurs in agricultural fields and along roadsides. *Salvia syriaca* L. was collected during its flowering stage from the province of Kırşehir during the 2023–2024 vegetation period. The plant material was collected from a location at an elevation of 1020 m in Kırşehir province (coordinates 39° 5′32.22′′N–34°10′25.1′′E). The plant material was collected as part of a study supported by the Scientific Research Projects Coordination Office of Kırşehir Ahi Evran University (project no: ZRT.A3.17.005), and the plant specimen herbarium was sent to the General Directorate of Plant Production (BÜGEM) and registered. The plant material was identified by Dr. Melih YILAR.The collected plants were shade-dried at room conditions in the Plant Protection Department Laboratory of Ahi Evran University, Faculty of Agriculture. The dried plant material was ground using an electric grinder and stored in the shade.

### 2.2 | Extraction Procedure

For biological activity studies, 100 g of ground plant material (aerial parts of plant) was placed in a 1-liter Erlenmeyer flask, and 600 mL of methanol was added. The mixture was subjected to extraction in a shaker at room temperature for 24 h. After extraction, the solution was filtered through filter paper, and the methanol was removed using a rotary evaporator at 32°C until solid matter was obtained. The remaining solid residue was dissolved in DMSO + water (5% DMSO, 95% water) to prepare a stock solution (Yılar et al. 2020), which was stored at +4°C until use.

For antioxidant tests, 200 mg of ground plant sample was used for each of the hexane, ethyl acetate, and methanol extracts. To each sample, 10 mL of hexane/chloroform (5/1), ethyl acetate/chloroform (5/1), and methanol/chloroform (5/1) mixtures were added, respectively. After vortexing, samples were kept in an ultrasonic bath at 30°C for 30 min. The obtained extracts were evaporated using a rotary evaporator and stock solutions were prepared at a concentration of 1 mg/mL. These solutions were stored at +4°C for use in antioxidant activity tests and total phenolic and flavonoid analyses. A standard curve was prepared using Trolox, BHA, and BHT.

### 2.3 | Determination of Phenolic Compounds of S. syriaca

Ultra-pure analytical standards (all ≥95% purity), including ferulic acid, *p*-coumaric acid, vanillin, rutin, (+)-catechin, caffeic acid, (−)-epicatechin, salicylic acid, gallic acid, *p*-hydroxybenzoic acid, resveratrol, oleuropein, rosmarinic acid, taxifolin, protocatechuic acid, protocatechuic aldehyde, and ellagic acid, were purchased from Sigma–Aldrich (St. Louis, MO, USA).

All solutions were prepared using analytical-grade reagents and ultrapure water. LC-MS grade methanol and chloroform were obtained from Merck, while HPLC-grade formic acid was supplied by J.T. Baker. Stock standard solutions (1000 mg $L^{-1}$ in methanol) were stored at 4 ± 2°C. Phenolic compounds were analyzed using external standard calibration.

The plant extract was dissolved in a methanol/chloroform mixture and sonicated for 1 h at room temperature, followed by centrifugation at 1500×g for 10 min. The supernatant was filtered through a 0.45 µm PVDF syringe filter and injected into the LC-MS/MS system. Phenolic compounds were quantified according to Kayir et al. (2023) and expressed as mg $kg^{-1}$ dry matter.

### 2.4 | DPPH-Free Radical Scavenging Activity

Conducted with modifications to Liyana–Pothirano's method (Liyana-Pathirana and Shahidi 2005). Extracts were diluted to 3 mL with ethanol and 1 mL of 0.26 mM DPPH was added. After 30 min in darkness, absorbance was measured at 517 nm. $IC_{50}$ values were calculated. A standard curve was prepared using Trolox, BHA, and BHT.

## 2.5 | Ferric-Reducing Power Activity (FRAP)

Modified from Oyaizu (1986). 0.25 mL extract + 1.25 mL phosphate buffer (pH 6.6) + 1.25 mL potassium ferricyanide (1%) were incubated at 50°C for 20 min. After cooling, 1.25 mL TCA (10%) and 0.25 mL $FeCl_3$ (0.1%) were added. Absorbance was read at 700 nm. Results are given as Trolox equivalents (TE).

## 2.6 | *Cupric Ion Reducing Antioxidant Capacity* (*CUPRAC*)

ML Extract Was Made up to 1 mL with Methanol. 1 mL Each of $CuCl_2$ (0.01 M), Neocuproine ($7.5 \times 10^{-3}$ M), and Ammonium Acetate Were Added. After 30 Min at Room Temperature, Absorbance Was Read at 450 nm. Results Were Expressed as Trolox Equivalent (μmol TE/Mg Extract) (Elmastas et al. 2018).

## 2.7 | Trolox Equivalent Antioxidant Capacity

Based on Re et al. (1999) ABTS and sodium persulfate solutions were mixed (1:2) and incubated in darkness for 6 h. Extracts were diluted with 0.1 M phosphate buffer (pH 7.4) to 3 mL, and then 1 mL ABTS solution was added. After 30 min at room temperature, absorbance was read at 734 nm. $IC_{50}$ was calculated.

## 2.8 | Total Phenolic Content

The total phenolic content was determined using the Folin–Ciocalteu reagent according to the method described by Singleton et al. (1999). In this procedure, 0.2 mL of the extract was mixed with 4.6 mL of distilled water, followed by the addition of 0.1 mL of Folin–Ciocalteu reagent and 0.3 mL of 2% $Na_2CO_3$ solution. After 2 h at room temp, absorbance was measured at 760 nm. Results expressed as gallic acid equivalents (mg GAE/g extract).

## 2.9 | Total Flavonoid Content

ML Extract + 4.8 mL Methanol + 0.1 mL $Al(NO_3)$ (10%) + 0.1 mL $NH_4CH_3COO$ (1 M). After Vortexing and 40 Min at Room Temp, Absorbance Was Read at 415 nm. Results Were Calculated as Quercetin Equivalents (mg QE/g Extract) (Chang et al. 2002).

## 2.10 | Allelopathy Assay

Filter papers were placed in two layers in 9 cm petri dishes. Seeds of the test plants (Triticum aestivum L., Rumex crispus L., and Taraxacum officinale F.H. Wigg) were distributed evenly (25 seeds per petri dish). By dissolving with methanol extract (DMSO + water); 1, 5, and 10 mg doses were obtained and applied, while distilled water was used for control. A total of 5 mL solution was added to each petri dish. Petri dishes were incubated at 25°C under 12 h light/12 h dark photoperiod for 4 weeks. At the end of this period, germination rate and root/shoot lengths were measured. The experiment was conducted in four replicates and repeated twice (Yılar et al. 2014).

## 2.11 | Antifungal Activity

The methanol extract was dissolved in an acetone–water mixture to prepare a stock solution. This was added to PDA (patato dextrose agar) medium cooled to 45–50°C to obtain final concentrations of 50, 100, 200, and 400 ppm (Onaran and Yılar 2012). As a control (Control −), fungi were grown on PDA without extract. A Thiram-based fungicide was used as positive control (Control +). Each PDA medium was poured into 60 mm petri dishes (10 mL per plate). Fungal pathogen cultures, previously grown for 7–10 days, were used to obtain 5 mm mycelial discs, which were placed on PDA plates containing the extract. The plates were incubated at 25 ± 1°C for 7 days. Mycelial growth diameters were measured with a digital caliper. Mycelial growth inhibition was calculated using the formula:

$$I = 100 \times (dc - dt)/dc$$

where I is the percent mycelial growth inhibition; dc is the growth in control; and dt is the growth in treatment (Pandey et al. 1982).

## 2.12 | Antibacterial Analysis

### 2.12.1 | Plant Pathogenic Bacteria Used in the Study

The study used *Clavibacter michiganensis* subsp. *michiganensis* (Cmm-PK-1) from vascular tissues of diseased tomato plants, *Pseudomonas syringae* pv. *tomato* (Pst) from diseased tomato leaves, and *Xanthomonas euvesicatoria* (Xe) from diseased pepper leaves in Tokat province. The isolates were stored in nutrient broth + glycerol at -20°C in the Plant Protection Department, Gaziosmanpasa University. Fresh 2-day cultures were prepared on Nutrient Agar (NA) incubated at 25 ± 2°C. Dilution series were made and suspensions were prepared at 1 × 106 CFU/mL.

### 2.12.2 | Analysis Methods

Plant extract was prepared at 0 (control) 500, 1000, 2000, and 4000 ppm and incorporated into autoclaved NA medium. Streptomycin, a commercial antibiotic, was used as a positive control. These were poured into 90 mm petri dishes, allowed to solidify, and inoculated with 100 μL of bacterial suspension (1 × 106 CFU/mL). Spread plating was done with a sterile glass rod. Controls were NA medium without extract. After 2 days of incubation at 25 ± 2°C, colonies were suspended in saline buffer, and optical density was measured at 600 nm. Inhibition (%) was calculated using Abbott's formula (Karman 1971):

$$\%\ \text{Inhibition} = [(\text{control} - \text{treatment})/\text{control}] \times 100$$

## 2.13 | Insecticidal Activity

*Sitophilus granarius* L. (Coleoptera: Curculionidae) was reared on whole wheat in 1 L jars. Adults were transferred to jars with one-third wheat for 1 week and removed to allow new adults to emerge. Adults < 1 week old were used. Acetone was used as control and extract doses of 5%, 10%, and 20% were prepared. 1 μL of extract was applied to each insect dorsally using a microsyringe. Treated insects were placed in 9 cm petri dishes containing 1 g wheat. Experiments were conducted in completely randomized design with 10 insects per replicate, five replicates, and repeated twice. Mortality was recorded at 24, 48, 72, and 96 h (Emsen et al. 2015).

### 2.14 | Statistical Analysis

The data obtained in this study were statistically evaluated. A value of $p \leq 0.05$ was accepted as significant in statistical calculations and comparisons. The data were analyzed by ANOVA test with 95% confidence intervals, and the differences between habitats were determined by Duncan and LSD in multiple comparisons with SPSS 22 Statistical Software.

## 3 | Results and Discussion

### 3.1 | Phenolic Composition of S. syriaca

LC-MS/MS analysis of *S. syriaca* methanol extract identified nine phenolic compounds, including phenolic acids and flavonoids (Table 1 and Figure 1). The dominant compound was ferulic acid (2850.430 ± 65.546 mg/kg DW), followed by rutin (2014.449 ± 3.972 mg/kg DW) and rosmarinic acid (82.501 ± 3.515 mg/kg DW).

Ferulic acid was identified as the most abundant constituent (2850.430 ± 65.546 mg/kg DW). Although rosmarinic acid or luteolin glycosides are typically reported as the major phenolics in many *Salvia* species (Rahman et al. 2007; Onder et al. 2022), the predominance of ferulic acid in *S. syriaca* suggests a distinct chemotaxonomic pattern. Given its well-established antioxidant and anti-inflammatory properties (Shehadeh et al. 2014) ferulic acid is likely a key contributor to the extract's biological activity.

Rutin was the second most abundant compound (2014.449 ± 3.972 mg/kg DW). Previous studies have reported moderate rutin levels in other *Salvia* species (Ben Farhat et al. 2009), whereas its high concentration in *S. syriaca* indicates an active flavonoid biosynthetic pathway and suggests a substantial contribution to the plant's antioxidant potential.

Although rosmarinic acid is considered one of the characteristic metabolites of the *Salvia* genus, it was detected at relatively low levels (82.501 ± 3.515 mg/kg DW). This may indicate a shift within the phenylpropanoid pathway favoring ferulic acid production over rosmarinic acid synthesis (Petersen 2013).

Overall, the phenolic profile of *S. syriaca* demonstrates that its biological potential is largely driven by ferulic acid and rutin. This distinctive distribution of phenolics is noteworthy both for its pharmacological relevance and its implications for the chemotaxonomic characterization of the species.

Previous studies have reported the phenolic composition of *Salvia syriaca*. In the methanol extract of *S. syriaca*, 13 phenolic compounds were identified, with the major constituents being rutin (9425 ± 163 µg/g extract), rosmarinic acid (629 ± 19 µg/g extract), apigenin (243 ± 5 µg/g extract), and ferulic acid (186 ± 3 µg/g extract) (Bahadori et al. 2017). Additionally, Fotovvat et al. (2019) identified several phenolic compounds in both aerial and root parts of the plant. Rosmarinic acid was found at (5.17 ± 0.46 mg/g DW) in the leaves and (2.68 ± 0.18 mg/g DW) in the roots; salvianolic acid B at (54.47 ± 2.00 mg/g DW) in the leaf and (2.60 ± 0.27 mg/g DW) in the root; salvianolic acid A at (1.20 ± 0.19 mg/g DW) in the leaf and (0.30 ± 0.12 mg/g DW) in the root; carnosic acid at (12.66±1.35 mg/g DW) in the leaf and (3.80 ± 0.28 mg/g DW) in the root; and caffeic acid at (4.69 ± 0.35 mg/g DW) in the leaf and (2.27 ± 0.61 mg/g DW) in the root.

### 3.2 | Total Phenolic and Flavonoid Content of *Salvia syriaca*

In the present study, the total phenolic and flavonoid contents of methanol, ethyl acetate, and hexane extracts of *S. syriaca* are summarized in Table 2. The extracts obtained using different solvents showed significant differences in total phenolic content. The highest total phenolic content was recorded in the ethyl acetate extract (37.42 mg GAE/g extract), followed by the methanol extract (34.07 mg GAE/g extract) and the hexane extract (14.40 mg GAE/g extract). Regarding the total flavonoid content, the highest amount was also observed in the ethyl acetate extract (23.29 mg QE/g extract) (Table 2).

In previous studies, the total phenolic content of *S. syriaca* methanol extract was reported as 51.2 ± 3.1 µg/mg, while the total flavonoid content was 40.51 ± 0.77 µg/mg (Iravani et al. 2019). Similarly, Karamian et al. (2014) reported the total phenolic content as 5.89 ± 0.26 mg/g DW and the total flavonoid content as 2.15 ± 0.28 mg/g DW in the methanol extract of *S. syriaca*. In the same study, the extract yield (W/W) was reported to be 22.5%.

### 3.3 | Antioxidant Activities

The DPPH radical scavenging activities of *S. syriaca* extracts are presented in Figure 2. The $IC_{50}$ values for the methanol, ethyl acetate, and hexane extracts were 55.46 ± 0.50 µg/mL, 70.53 ± 0.51 µg/mL, and 126.91 ± 0.74 µg/mL, respectively. When compared with standard antioxidants such as BHT ($IC_{50}$ = 10.85 ± 0.34 µg/mL), BHA ($IC_{50}$ = 4.73 ± 0.14 µg/mL), and Trolox ($IC_{50}$ = 4.49 ± 0.08 µg/mL), both the methanol and ethyl acetate extracts exhibited a notably high DPPH radical scavenging capacity, indicating substantial antioxidant potential (Figure 1). On the other hand, the hexane extract demonstrated relatively weak DPPH scavenging activity.

### 3.4 | Reducing Power Capacity of the Extracts

The reducing power of a compound is considered a key indicator of its antioxidant potential. The iron reducing capacity of the *S. syriaca* extracts is illustrated in Figure 3. When compared with standard antioxidants BHT (4.73 ± 0.17 µmol TE/mg extract) and BHA (5.63 ± 0.23 µmol TE/mg extract), the ethyl acetate extract demonstrated the highest reducing activity (1.13 ± 0.04 µmol TE/mg extract), followed by methanol (0.82 ± 0.002 µmol TE/mg extract) and hexane extracts (0.48 ± 0.001 µmol TE/mg extract). These findings indicate that the ethyl acetate extract of *S. syriaca* possesses a notable iron-reducing capacity, which may be attributed to its phenolic composition and related antioxidant constituents.

### 3.5 | ABTS Cation Radical Scavenging Activity

The ABTS cation radical scavenging activity results of the *Salvia syriaca* extracts are presented in Figure 4. Among the extracts, the methanol extract exhibited the highest scavenging activity with an $IC_{50}$ value of 16.76 ± 0.55 µg/mL, followed by ethyl acetate ($IC_{50}$ = 16.99 ± 0.01 µg/mL) and hexane ($IC_{50}$ = 116.58 ± 0.14 µg/mL). When compared with standard antioxidants such as BHT ($IC_{50}$ = 4.71 ± 0.03 µg/mL), BHA ($IC_{50}$ = 3.86 ± 0.03 µg/mL), and Trolox ($IC_{50}$ = 6.92 ± 0.07 µg/mL), both the methanol and ethyl acetate extracts of *S. syriaca* demonstrated notable ABTS

**TABLE 1** | Phenolic compound content (mg/kg dry weight) in *S. syriaca* methanol extract.

| No | RT | Phenolic compound | Formula | Parent ion, *m/z* | MS/MS, CE | Ionization mode | LOD, mg $L^{-1}$ | LOQ, mg $L^{-1}$ | Amount, ppm |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 10.05 | Gallic acid | $C_7H_6O_5$ | 169.7 | 80.5 (25)–126.2 (16) | Neg | 0.061 | 0.203 | <LOD |
| 2 | 12.13 | Protocatechuic acid | $C_7H_6O_4$ | 136.9 | 110.4 (17)–92.5(27) | Neg | 0.049 | 0.162 | <LOD |
| 3 | 13.16 | protocatechuic aldehyde | $C_7H_6O_3$ | 153.808 | 92.25 (25)–108.2 (25) | Neg | 0.026 | 0.087 | <LOD |
| 4 | 13.24 | Catechin | $C_{15}H_{14}O_6$ | 289.2 | 203.9 (22)–245.7 (17) | Pos | 0.068 | 0.227 | 0.998 ± 0.097 |
| 5 | 14.72 | Epicatechin | $C_{15}H_{14}O_6$ | 291.5 | 123.3 (15)–139.3 (16) | Pos | 0.045 | 0.151 | 9.673 ± 0.909 |
| 6 | 15.27 | Caffeic acid | $C_9H_8O_4$ | 179.7 | 135.2 (27)–136.2 (18) | Neg | 0.047 | 0.157 | 9.960 ± 0.926 |
| 7 | 15.87 | Vannilin | $C_8H_8O_3$ | 150.91 | 92.3 (23)–136.1(16) | Neg | 0.023 | 0.076 | 5.130 ± 0.582 |
| 8 | 16.68 | Taxifolin | $C_{15}H_{12}O_7$ | 303 | 126.2 (23)–285.5 (15) | Neg | 0.058 | 0.194 | <LOD |
| 9 | 17.00 | P-coumaric acid | $C_9H_8O_3$ | 163.9 | 94.3 (33)–120.2 (17) | Neg | 0.116 | 0.387 | <LOD |
| 10 | 17.19 | Ferulic acid | $C_{10}H_{10}O_4$ | 193.35 | 89.4 (30)–177.4 (7) | Neg | 0.061 | 0.204 | 2850.430 ± 65.546 |
| 11 | 17.82 | Rosmarinic Acid | $C_{18}H_{16}O_8$ | 359.18 | 134.3 (44)–162.2 (20) | Neg | 0.029 | 0.095 | 82.501 ± 3.515 |
| 12 | 18.00 | Oleuropein | $C_{25}H_{32}O_{13}$ | 539.1 | 275.8 (22)–377–5 (16) | Neg | 0.050 | 0.167 | <LOD |
| 13 | 18.12 | P-hydroxybenzoic acid | $C_7H_6O_3$ | 137.9 | 66.6 (38)–94.6 (17) | Neg | 0.031 | 0.104 | 8.356 ± 0.667 |
| 14 | 18.13 | Salicylic acid | $C_7H_6O_3$ | 137.14 | 65.51 (35)–93.26 (18) | Neg | 0.030 | 0.099 | 7.670 ± 0.569 |
| 15 | 18.26 | Rutin | $C_{27}H_{30}O_{16}$ | 609.37 | 300.6 (38)–301.7 (34) | Neg | 0.007 | 0.024 | 2014.449 ± 23.972 |
| 16 | 18.45 | Resveratrol | $C_{14}H_{12}O_3$ | 228.98 | 107.2 (22)–135.1 (14) | Pos | 0.030 | 0.099 | <LOD |
| 17 | 19.47 | Ellagic acid | $C_{14}H_{10}O_{10}$ | 300.9 | 229.7 (28) | Neg | 0.087 | 0.289 | <LOD |

*Note:* Parent ion (*m/z*): Molecular ions of the standard compounds (LOD, limit of detection; LOQ, Limit of Quantification, Neg: Negative ionization mode Pos: Positive İonasitaion Mode, Rt: Retention Time).

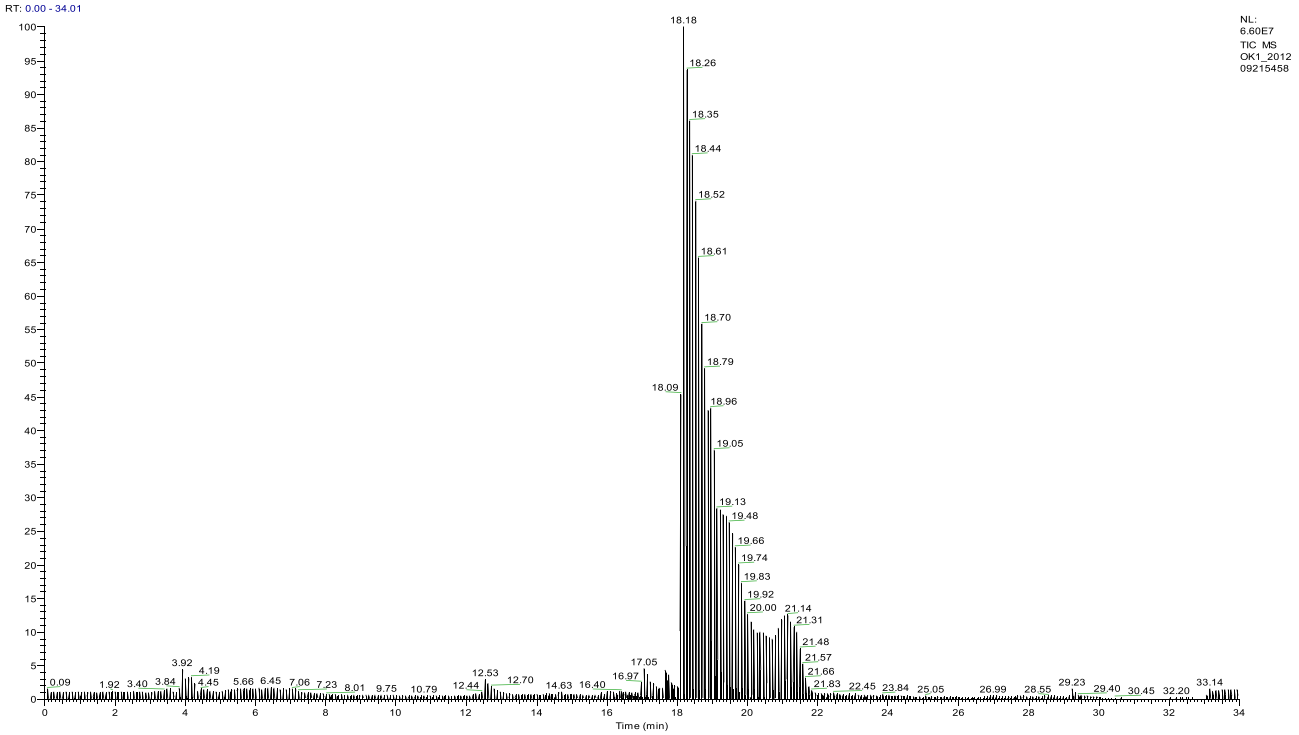


**FIGURE 1** | Chromatograms of standard phenolic compounds in LC-MS/MS analyses.

**TABLE 2** | Total phenolic and total flavonoids in *Salvia syriaca*.

| Solvent of extracts | Total phenolic content, mg GAE/g extracts | Total flavonoid content, mg QE/g extracts |
|---|---|---|
| **Ethyl acetate** | $37.42^{a} \pm 0.26$ | $23.29^{a} \pm 0.52$ |
| **Methanol** | $34.07^{b} \pm 0.31$ | $21.71^{b} \pm 0.34$ |
| **Hexane** | $14.40^{c} \pm 0.17$ | $7.91^{c} \pm 0.20$ |

*Note:* Means in the same column with different letters are significantly different at $p < 0.05$, Duncan's test).

cation radical scavenging activity. In contrast, the hexane extract exhibited significantly lower scavenging potential.

## 3.6 | CUPRAC

The CUPRAC results of *S. syriaca* extracts are presented in Figure 5. When compared with standard antioxidant compounds such as BHT (5.68 ± 0.15 μmol TE/mg extract) and BHA (11.18 ± 0.35 μmol TE/mg extract), the methanol extract exhibited the highest reducing capacity with a value of 6.89 ± 0.31 μmol TE/mg extract. This was

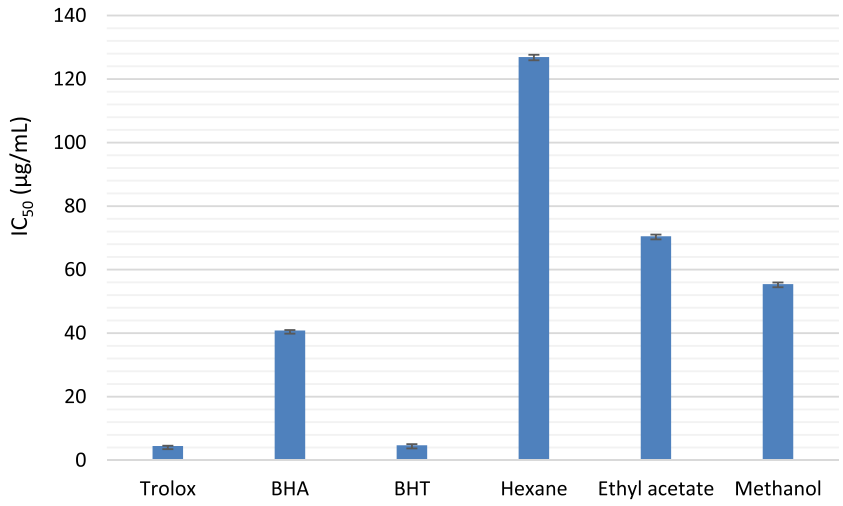


**FIGURE 2** | *S. syriaca extracts*: DPPH-free radical activity.

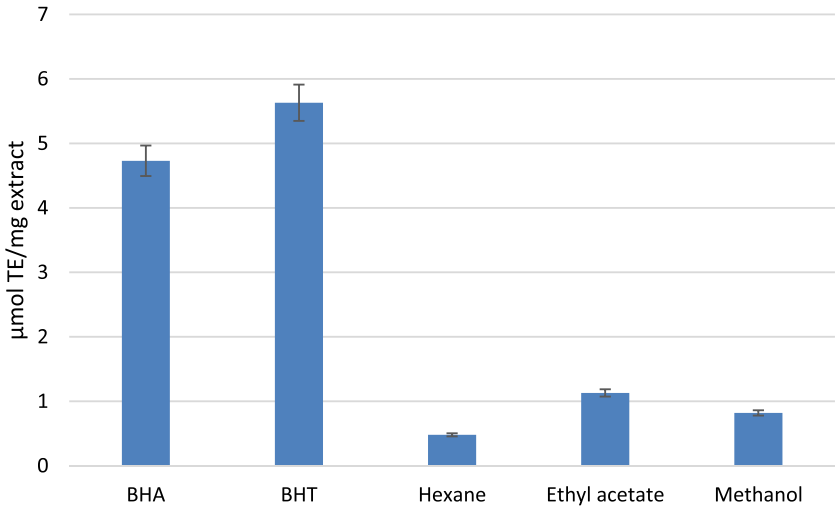


**FIGURE 3** | *S. syriaca* extracts: reducing power activity.

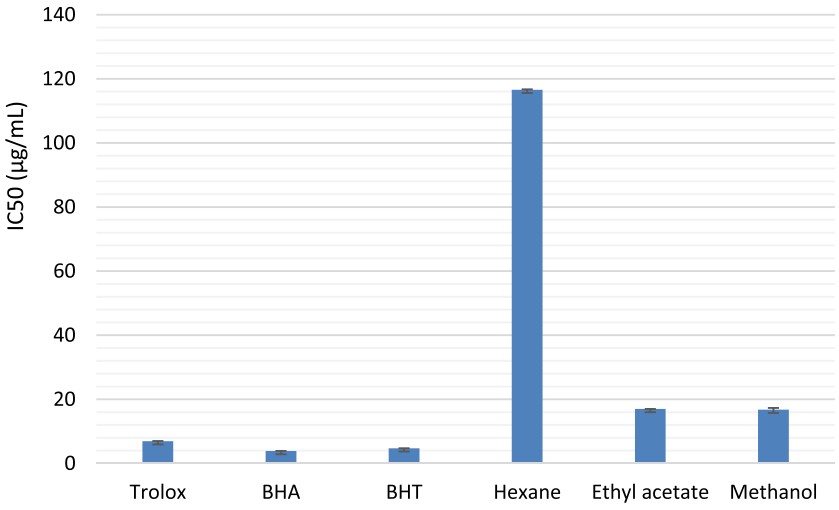


**FIGURE 4** | *S. syriaca extracts*: ABTS cation radical scavenging.

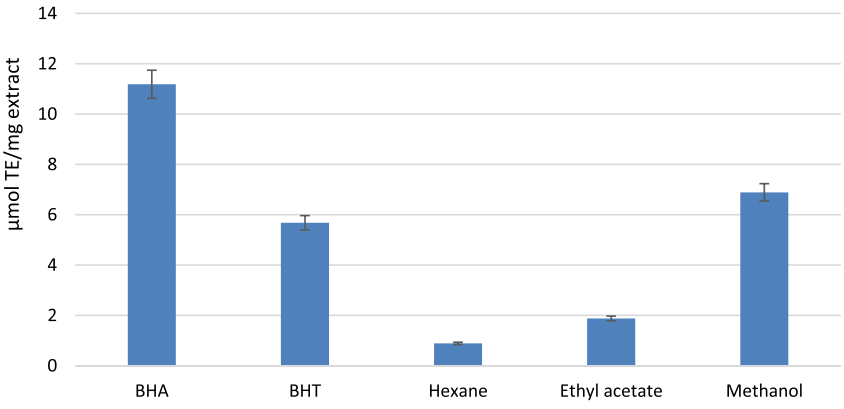


**FIGURE 5** | *S. syriaca extracts*: C copper reducing power activity (CUPRAC).

followed by the ethyl acetate extract (1.88 ± 0.04 µmol TE/mg extract) and the hexane extract (0.89 ± 0.08 µmol TE/mg extract). These results demonstrate that the methanol extract of *S. syriaca* possesses a noteworthy copper-reducing power, highlighting its potential as a natural antioxidant agent.

## 3.7 | Biological Activity of S. syriaca

### 3.7.1 | Allelopathic Activity

The methanol extract of *S. syriaca* showed negative effects on the germination, root, and shoot development of weed species

**TABLE 3** | Effect of *S. syriaca* methanol extract on test plants.

| | *Taraxacum officinale* | | | *Rumex crispus* | | |
|---|---|---|---|---|---|---|
| **Dose, ppm** | **Germination, %** | **Root, cm** | **Shoot, cm** | **Germination, %** | **Root, cm** | **Shoot, cm** |
| **Control** | $88.00^{a*} \pm 2.3$ | $6.98^{a} \pm 1.1$ | $10.65^{a} \pm 1.4$ | $100.00^{a} \pm 2.3$ | $16.49^{a} \pm 1.1$ | $18.73^{a} \pm 1.4$ |
| **1000** | $12.00^{b} \pm 2.3$ | $0.21^{b} \pm 0.02$ | $1.35^{b} \pm 0.1$ | $93.3^{a} \pm 2.3$ | $11.65^{b} \pm 0.02$ | $17.66^{a} \pm 0.1$ |
| **2000** | $0.00^{c} \pm 0.0$ | $0.00^{b} \pm 0.0$ | $0.00^{b} \pm 0.0$ | $65.0^{b} \pm 0.0$ | $3.57^{c} \pm 0.0$ | $13.78^{b} \pm 0.0$ |
| **3000** | $0.00^{c} \pm 0.0$ | $0.00^{b} \pm 0.0$ | $0.00^{b} \pm 0.0$ | $60.0^{b} \pm 0.0$ | $0.82^{c} \pm 0.0$ | $2.38^{c} \pm 0.0$ |

*Note:* *Means in the same column with different letters are significantly different at $p < 0.05$, Duncan's test.

(*Taraxacum officinale* and *Rumex crispus*), and the degree of this inhibition varied depending on the extract dose and plant species. These effects were found to be statistically significant ($p < 0.05$) (Table 3).

The 2000 ppm dose of the methanol extract of *S. syriaca* completely inhibited germination and seedling development of *T. officinale*. *R. crispus* showed more tolerance; the same dose inhibited germination, root, and shoot growth by 40%, 95.03%, and 87.29%, respectively. These results suggest that *S. syriaca* could be used for controlling these weeds.

Research on the phytotoxic and allelopathic effects of *S. syriaca* is relatively limited. Existing studies have demonstrated that this species exhibits significant allelopathic effects on various cultivated and weed plant species. Qasem and Abu-Irmaileh (1985) reported that extracts obtained from the shoots and rhizomes of *S. syriaca* reduced wheat germination and seedling growth under greenhouse and laboratory conditions. In another study, Qasem (2001) evaluated the allelopathic potential of the essential oils, leaf exudates, root secretions, and dried shoot residues of *S. syriaca* under greenhouse and laboratory conditions. The bioassays were conducted on cabbage (*Brassica oleracea* L. var. Capitata cv. Pronzwik), carrot (*Daucus carota* L. cv. Natus), cucumber (*Cucumis sativus* L. cv. Beithalpha), squash (*Cucurbita pepo* L. cv. Byrouti), onion (*Allium cepa* L. cv. Texas Early Grana), pepper (*Capsicum annuum* L. cv. Red Common), and tomato (*Lycopersicon esculentum* Mill. cv. Special Back). The results indicated that the effects varied depending on the plant part used, showing differential levels of allelopathic activity on the test species. Mousavian and Skandari (2018) demonstrated that methanol extracts of *S. syriaca* negatively affected seed germination, seed formation, and seedling growth of *Portulaca oleracea* L. Similarly, Yılar and Bayar (2018) reported that both methanol and hexane extracts of *S. syriaca* exhibited phytotoxic effects on seed germination as well as root and shoot growth of *Lactuca sativa* L., *Lepidium sativum* L., and *Triticum vulgare*.

### 3.7.2 | Antifungal Activities

The antifungal activity values (mycelial growth inhibition = MGI) of the methanol extract obtained from *S. syriaca* against *Phytophthora infestans*, *Cylindrocarpon destructans*, and *Fusarium oxysporum* f. sp. *cucumerinum* are presented in Table 4. The methanol extract inhibited the mycelial growth of the tested fungi at varying rates depending on the dose, although complete (100%) inhibition was not observed at any concentration. At the highest dose (6000 ppm), mycelial growth inhibition was determined as 21.75% for *P. infestans*, 25.75% for *C. destructans*, and 49.25% for *F. oxysporum* f. sp. *cucumerinum*. These results indicate that *F. oxysporum* f. sp. *cucumerinum* was the most sensitive pathogen to the plant extract, whereas *P. infestans* was the most tolerant.

**TABLE 4** | Percent inhibition of fungal mycelial growth by *S. syriaca* methanol extract.

| **Dose, ppm** | ***Cylindrocarpon destructans*** | ***Phytophthora infestans*** | ***Fusarium oxysporum f. sp. cucumerinum*** |
|---|---|---|---|
| **Control+** | $100.0^{a*} \pm 0.00$ | $100.0^{a*} \pm 0.00$ | $\mathbf{100.0^{a*} \pm 0.00}$ |
| **Control−** | $0.00^{e} \pm 0.00$ | $0.00^{c} \pm 0.00$ | $0.00^{d} \pm 0.00$ |
| **2000** | $15.26^{d} \pm 2.03$ | $18.50^{b} \pm 1.30$ | $38.68^{c} \pm 1.31$ |
| **4000** | $21.58^{c} \pm 0.75$ | $20.02^{b} \pm 0.71$ | $46.01^{b} \pm 0.62$ |
| **6000** | $25.75^{b} \pm 1.92$ | $21.16^{b} \pm 1.30$ | $49.25^{b} \pm 3.13$ |

*Note:* *Means with different letters in each column differ significantly by ANOVA ($p < 0.05$).

Similar antifungal activities of sage essential oils and extracts have been reported in literature (Yilar and Bayar 2018; Yilar et al. 2020). *S. syriaca* methanol and hexane extracts also demonstrated antifungal activity against *Rhizoctonia solani*, *Alternaria solani*, *Fusarium oxysporum* f.sp. *radicis lycopersici*, and *Verticillium dahliae* (Yilar and Bayar 2018). In another study, the essential oil of *S. syriaca* was reported to be effective against *Candida albicans* (Karataş and Ertekin 2010). In the present study, the methanol extract of *S. syriaca* was found to exert a considerable inhibitory effect on the mycelial growth of the tested plant pathogens. Furthermore, it has been reported that *S. syriaca* shows a comparable level of activity against the pathogens *Candida albicans* and *Candida glabrata* (Demirpolat 2023).

### 3.7.3 | Antibacterial Activities

The antibacterial activity of *S. syriaca* methanol extract against *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), *Pseudomonas syringae* pv. *tomato* (Pst), and *Xanthomonas euvesicatoria* (Xe) is summarized in Table 5. The inhibitory effect increased with the dose. At 2000 and 4000 ppm, complete inhibition (100%) was observed. At 1000 ppm, Cmm and Pst were inhibited by 75% and 81%, respectively. No significant inhibition occurred at 500 ppm.

There are also studies investigating the activity of *S. syriaca* essential oils and extracts against bacteria; however, these pathogens are mostly human pathogenic bacteria. These studies have reported that *S. syriaca* possesses antibacterial properties. The essential oil of *S. syriaca* exhibited a low level of activity against

**TABLE 5** | Antibacterial effect of *S. syriaca* methanol extract.

| Dose, ppm | Inhibition rate, % | | |
|---|---|---|---|
| | ***Clavibacter michiganensis* subsp. *michiganensis*** | ***Pseudomonas syringae* pv. *tomato*** | ***Xanthomonas euvesicatoria*** |
| **Control −** | 0.0[a*] | 0.0[a] | 0.0[a] |
| **Control+** | 100[d] | 100[d] | 100[d] |
| **500** | 23.0[b] | 30.0[b] | 0.0[a] |
| **1000** | 75.0[c] | 81.0[c] | 42.0[b] |
| **2000** | 100.0[d] | 98.0[c] | 100.0[c] |
| **4000** | 100.0[d] | 100.0[c] | 100.0[c] |

*Note:* *Means with different letters in each column differ significantly by ANOVA ($p < 0.05$).

*Escherichia coli*, *Klebsiella pneumoniae*, *Staphylococcus aureus*, and *Bacillus megaterium* (Demirpolat 2023). Similarly, antibacterial activity was observed against *E. coli* K12, *Pseudomonas aeruginosa*, *S. aureus*, *Streptococcus pyogenes*, and *Bacillus subtilis* (Karataş and Ertekin 2010).

### 3.7.4 | Insecticidal Activities

This study evaluated the insecticidal effectiveness of methanol extract from *S. syriaca* against *S. granarius* at several concentrations (5%, 10%, and 20%) and durations of exposure (24, 48, 72, and 96 h). Studies have indicated that the extract considerably increased mortality rates in a dose- and time-dependent way (Figure 6).

At the end of the 24th h, the highest rate of mortality was found at 20% dosage ($F_{3,36} = 66.28$) ($p < 0.05$). The mortality rate at 10% and 20% doses was found to be more than 50% at the end of the 48th hour ($F_{3,36} = 76.21$; $p < 0.05$). The mortality rates at the 72nd and 96th hours were similar, too. At the end of 96 h, a mortality rate of 50% was recorded at a 5% dose, 68% at a 10% dose, and 84% at a 20% dose ($F_{3,36} = 91.82$) ($p < 0.05$) (Figure 5).

*Sitophilus granarius* is an important pest of cereal grains. Management of this insect is highly difficult, especially around storage areas. Due to the difficulty related to fumigant application, it is important to find alternative control strategies. Plant-based insecticides are one of these alternate control strategies. In recent years, research in this area has expanded. Parventi et al. (2021) examined the topical effectiveness of various extracts of *Humulus lupulus* L. against *S. granarius*, obtaining 100% mortality at a dose of 75 μg per adult for the *n*-hexane extract. Emsen et al. (2015) examined extracts obtained from *Lecanora muralis* (Schreb.) Rabenh. *Letharia vulpina* (L.) Hue and *Peltigera rufescens* (Weiss) Humb demonstrated a 100% mortality rate against *S. granarius* at 96 h at the highest dose. Jawalkar et al. (2021) tested the efficacy of *Vitex negundo*, *Xanthium strumarium*, *Caesalpinia bonduc*, *Mucuna pruriens*, *Moringa oleifera*, and *Tagetes erecta* against *S. granarius*, showing that a 40% dose of the six plant extracts was effective. The results showed that a 40% dose of all plant extracts significantly increased adult mortality rates, which varied from 70% to 100% (mean 88.3%) using methanol + n-hexane and 90% to 100% mortality (mean 96.7%) with acetone + petroleum ether. This study showed results that confirmed previous studies. The methanol extract of *Salvia syriaca* demonstrated efficacy against *S. granarius*, with mortality rates increasing in correlation with higher doses and the duration of exposure to them.

*Salvia*, one of the largest genera of the Lamiaceae family, comprises nearly 1000 species and includes medicinal, aromatic, culinary, and ornamental plants. These species have widespread applications in pharmaceuticals, food, and cosmetics industries (Luca et al. 2023). Notably, the Lamiaceae family especially the genus *Salvia* is recognized as a rich source of phenolic compounds. This genus contains various plant-derived bioactive substances that are used in medicine, dietary supplements, food additives, and as components in cosmetics and perfumes (Bahadori et al. 2015; Bahadori et al. 2017).

The biological properties of *Salvia* species are primarily associated with their anti-inflammatory, antiproliferative, antimicrobial, antimutagenic, antiangiogenic, and neuroprotective effects. Many of these activities are attributed to phenolic compounds due to their potent free radical scavenging and metal ion chelating capacities. The antioxidant properties of phenolic compounds are closely related to their molecular structures specifically, the presence and number of hydroxyl groups, and the influence of conjugation and resonance effects.

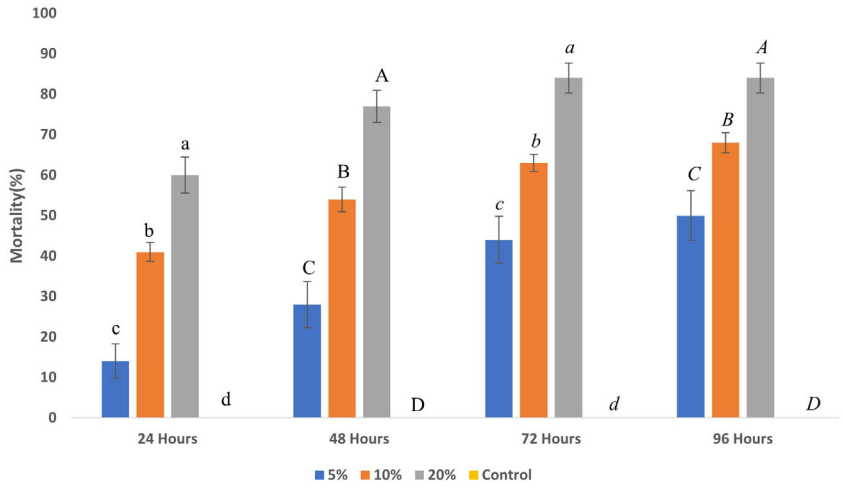


**FIGURE 6** | The effect of *S syriaca* extracts on *Sitophilus granarius*.

Due to their potential biological activities against pathogenic microorganisms, phenolic compounds are increasingly incorporated into food, pharmaceutical, healthcare, and surface-cleaning products to treat and prevent various microbial infections (Sauceda et al. 2017). In particular, phenolics found in the *Salvia* genus are regarded as important natural compounds due to their broad pharmacological activities, including antioxidant, anti-inflammatory, anticancer, and antimicrobial effects (Bahadori et al. 2017). Extensive studies have been conducted to identify and characterize these potential bioactive compounds in *Salvia* species, which are known to be rich in terpenoids (especially di- and triterpenoids), phenolic acid derivatives, and flavonoids. In this context, the present study aimed to determine the chemical composition and biological activity of *Salvia syriaca*, a species naturally distributed in the flora of Turkey.

## 4 | Conclusion

The results obtained from this study demonstrate that *Salvia syriaca* possesses promising *in vitro* antioxidant properties. It has been revealed that *S. syriaca* is rich in important phenolic compounds that can directly contribute to its antioxidant potential. The methanol extract of *S. syriaca* exhibited allelopathic, antifungal, antibacterial, and insecticidal activities, which are believed to be associated with its chemical constituents. These findings suggest that *S. syriaca* could serve as a potential natural alternative to synthetic chemicals in the management of weeds, plant pathogenic fungi and bacteria, as well as insect pests. Particularly in the context of organic agriculture whose importance continues to grow both globally and in Turkey, the species shows potential for application in commonly cultivated crops.

### Author Contributions

Dr. Melih YILAR and Dr. Yusuf Bayar collected the plant material together and planned the study together. Dr. Melih YILAR conducted the allelopathy study, Dr. Yusuf BAYAR conducted the antifungal part, Dr. Sabriye BELGÜZAR conducted the antibacterial part, Dr. Hayriye Didem SAĞLAM ALTINKÖY conducted the insecticidal activity, and Dr. Nusret GENÇ conducted the antioxidant activity. The article was written and reviewed by all authors.


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No funding was received for conducting this study.


### Conflicts of Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

### Data Availability Statement

Data will be made available on request.

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