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RESEARCH ARTICLE

Integrated Analysis of Volatile and Phenolic Profiles and Biological Activities of *Satureja hortensis* From Erzincan

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ABSTRACT

Satureja hortensis, widely used in traditional medicine and culinary applications, is a valuable aromatic plant rich in essential oils (Eos) and bioactive compounds. This study comprehensively evaluated the phytochemical composition and biological properties of *S. hortensis* grown in Erzincan, Turkey. The EO and four solvent-based extracts (ethyl acetate, butanol, water extract (WE), and water fraction) were analyzed. GC–MS/MS identified 34 volatile compounds (98.76%), with thymol (40.83%), carvacrol (19.77%), γ-terpinene (16.71%), and *p*-cymene (12.06%) as major constituents. LC-ESI-MS/MS revealed phenolic compounds, with hesperidin, rutin, and caffeic acid as major constituents in ethyl acetate and butanol fractions. Antioxidant activity was determined by DPPH, ferric reducing antioxidant power (FRAP), TPC, and TFC assays. The ethyl acetate showed the strongest DPPH scavenging (17.25 ± 2.34 µg/mL), highest TPC (79.24 ± 0.59 mg gallic acid equivalent /g), and moderate FRAP activity (96.86 ± 1.06 mg TE/g), while the WE had the highest flavonoid content (48.21 ± 1.56 mg QE/g). The EO exhibited potent antimicrobial activity (MIC 31.25–250 µg/mL; maximum inhibition zones 50 mm) against *A. niger*, *C. albicans*, and *A. alternata*. HPLC-DAD quantified rosmarinic acid, highest in ethyl acetate extract (580.93 ± 1.12 mg/g). Pearson correlation analyses demonstrated strong positive associations between phenolic content and biological activities, supporting the pharmaceutical, cosmetic, and industrial potential of *S. hortensis*.

Key Points

- Comprehensive GC-MS/MS and LC-MS/MS profiling of *Satureja hortensis* essential oil and solvent-based extracts from Erzincan, Türkiye, revealed thymol, carvacrol, hesperidin, and rutin as major bioactive constituents, alongside a diverse phenolic profile.
- HPLC-DAD quantification showed that the ethyl acetate fraction contained the highest rosmarinic acid concentration (580.93 ± 1.12 mg/g extract), consistent with its elevated total phenolic content and strong antioxidant activity in DPPH and FRAP assays.
- The essential oil exhibited potent antibacterial and antifungal activity, against *Staphylococcus aureus*, *Escherichia coli*, *Aspergillus niger*, and *Candida albicans*, for certain tested strains comparable or superior to standard antimicrobial agents.
- Principal component analysis (PCA) and Pearson correlation analysis provided a multidimensional evaluation, revealing strong positive associations between rosmarinic acid content, phenolic levels, and antioxidant/antimicrobial activities.
- The observed bioactivities are consistent with the high affinity of phenolic acids for semi-polar solvents, highlighting the importance of solvent selection and chemometric approaches in phytochemical–bioactivity correlation analyses.
- Findings support the potential application of *Satureja hortensis* essential oil and extracts as natural sources of antioxidant and antimicrobial agents for pharmaceutical, food preservation, and agricultural uses.

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1 | Introduction

Plants produce a wide variety of secondary metabolites that play significant roles in their adaptation to environmental stresses and interactions with pathogens. Beyond their ecological functions, these metabolites, particularly essential oils (EOs) and phenolic compounds, have long been valued for their pharmacological properties (Jan et al. 2021). Increasing concerns regarding the environmental persistence, toxic residues, and resistance-inducing effects of synthetic pesticides and antibiotics have reinforced the search for natural, plant-derived alternatives (Souto et al. 2021). Thus, aromatic and medicinal plants are considered promising sources of bioactive molecules with applications in food preservation, pharmaceuticals, and cosmetics.

Among aromatic plants, members of the Lamiaceae family are known for their volatile terpenoids and polyphenolic compounds. Within this family, the genus *Satureja*, with about 200 species across the Mediterranean Basin, West Asia, North Africa, the Canary Islands, and South America (Kavcı et al. 2022), has received considerable attention for EOs rich in phenolic monoterpenes (thymol, carvacrol) and extracts containing phenolic acids and flavonoids such as rosmarinic acid (RA), caffeic acid, rutin, and hesperidin. These constituents contribute to antioxidant, antimicrobial, antifungal, anti-inflammatory, and cytoprotective activities, supporting both traditional uses and pharmacological value (Azaz et al. 2002; Güllüce et al. 2003; Siavash Saei-Dehkordi et al. 2012; Kerbouche et al. 2013). In Turkey, 15 *Satureja* species, five endemic, reflect the country's rich biodiversity and role in preserving this genus (Karakoç et al. 2020; Kavcı et al. 2022).

In this genus, SH, locally known as 'Koç Otu' in Eastern Anatolia, is an annual aromatic herb (10–25 cm) with branched stems, linear leaves, and small white to pale pink flowers, traditionally used as a spice and for gastrointestinal, muscular, and infectious disorders (Hassanzadeh et al. 2016; Tepe and Cilkiz 2016). Phytochemical analyses have revealed that its EOs contain carvacrol, thymol, γ-terpinene, and p-cymene, while alcoholic extracts are rich in RA, caffeic acid, and flavonoids (Hamidpour et al. 2014). RA, widely found in Lamiaceae including SH, has strong antioxidant, antimicrobial, anti-inflammatory, and antiviral activities through radical scavenging, metal chelation, and modulation of inflammatory pathways (Guan et al. 2022; Noor et al. 2022). Quantitative RA determination is crucial for establishing the relationship between concentration and bioactivity and for standardizing extracts in therapeutic and food applications, yet detailed profiling across solvent fractions and under varying conditions remains scarce.

Although SH and related *Satureja* species have been investigated, comprehensive research on their chemical composition and biological activities, especially regarding regional and ecological variations, remains limited. The content of EOs and phenolics is strongly influenced by factors such as origin, altitude, climate, soil, cultivation, and harvest time, which can markedly affect bioactivity (Kumar et al. 2023). This highlights the need for region-specific studies to better evaluate therapeutic potential. Erzincan, a floristic transition zone between the Europe-Siberia and Iran-Turan regions, has microclimatic heterogeneity, diverse geology (serpentine, limestone), and a continental climate with cold winters and hot summers (Korkmaz and Karakuş 2015; Özbek et al. 2018; Kupe et al. 2021). Such stress conditions often stimulate secondary metabolite production in aromatic plants. However, data on SH from Erzincan are still scarce, limiting knowledge of its pharmacological potential and possible applications in biotechnology and pharmaceutical sciences.

This study aimed to perform a comprehensive phytochemical and biological evaluation of SH EO and solvent-based extracts from Erzincan. Solvents of varying polarity were used to recover a broad spectrum of phenolics, including RA and caffeic acids, hesperidin, and rutin, supporting consistent associations between compounds and bioactivities. EO composition was analyzed by GC–MS/MS, phenolic profiling by LC-ESI-MS/MS, and RA quantified by HPLC-DAD. Antioxidant potential was examined using DPPH, ferric reducing antioxidant power (FRAP), TPC, and TFC assays, while antimicrobial and antifungal activities were assessed using disc diffusion and MIC methods. Pearson correlation analysis revealed relationships between chemical profiles and biological effects. This integrated approach highlights the therapeutic relevance of S. hortensis, with solvent polarity as a key factor in bioactive compound recovery, and supports its pharmaceutical and functional potential, particularly in ecologically similar regions.

2 | Materials and Methods

2.1 | Plant Material

The fresh aerial parts of SH (Herbarium number: EBYU-0000039) were collected in limited quantities from multiple plants within the natural population at Erzincan Binali Yıldırım University during their flowering period in August 2022 to prevent damage to natural habitats. The plant was taxonomically identified by Prof. Dr. Ali Kandemir from the Faculty of Arts and Sciences, Department of Biology, Erzincan Binali Yıldırım University. Voucher specimens have been deposited in the EBYU Herbarium.

2.2 | Extraction Procedure

Fresh aerial parts of SH (500 g) were suspended in 2 L of distilled water and subjected to hydrodistillation for 4 h using a Neo–Clevenger apparatus, which is a modified form of the classical Clevenger recommended by the European Pharmacopeia, designed with a longer and narrower graduated collection arm that enables more accurate measurement of small oil volumes and reduces evaporation losses. The obtained EO was separated, dried over anhydrous sodium sulfate, and stored in amber vials at 4°C until chemical composition analysis and bioassays were performed. Following hydrodistillation, the residual aqueous phase in the distillation flask was collected. An aliquot was reserved as the water extract (WE). The remaining portion was subjected to successive liquid–liquid extraction, first with ethyl acetate and subsequently with n-butanol, to obtain the corresponding EtOAc and BuOH fractions. After completion of these extractions, the residual aqueous phase was collected and designated as the water fraction (WF). The WE and WF were lyophilized, while the EtOAc and BuOH fractions were concentrated in a rotary evaporator. All fractions were stored at 4°C until subsequent phytochemical and biological activity analyses. The sequential use of ethyl acetate followed by n-butanol after hydrodistillation is consistent with standard fractionation approaches in natural product research, which are widely applied to separate phenolic compounds of varying polarity.

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2.3 | GC–MS Analysis of EOs

GC–MS/MS analysis of EO was performed using a previously described method with minor modifications (Bayar et al. 2023; Tüfekçi et al. 2023). A Thermo Scientific GC–MS instrument with a Thermo Trace 1310 MS detector and an HP-5 MS capillary column (30 m length, 0.25 mm diameter, 0.25 μm film thickness) was used for GC–MS analysis of the EOs. The oven temperature was initially 60°C, increased to 200°C at 3°C/min, then to 240°C at 5°C/min, and held for 5 min. The injector split ratio was 1:20, and its temperature was 280°C. MS spectra were recorded using electron impact at 70 eV. Linear retention indices were determined by comparison with a reference n-alkane series (C8–C30) under identical conditions. Identification was confirmed by MS libraries (Wiley 275, NIST 08) and scientific literature. The percentage composition of identified components was calculated by normalizing peak areas from the FID chromatogram without correction factors.

2.4 | LC-ESI-MS/MS Conditions

An Agilent 1260 Infinity II LC System coupled with an Agilent 6460 Triple Quadrupole Mass Spectrometer was used for quantitative analysis of phytochemicals. Chromatographic separation was performed using an Agilent Poroshell 120 EC-C18 column (100 mm × 3.0 mm, 2.7 μm) at 40°C. The mobile phase consisted of water with 5mM ammonium formate (A) and acetonitrile with 0.1% formic acid (B). The gradient elution protocol was as follows: 3% B for 0–3 min, 50% B for 4–12 min, 90% B for 13–21 min, and 3% B for 22–25 min. The flow rate was 0.5 mL/min; the total analysis time was 25 min. Mass analysis was conducted in both ion modes using multiple reaction monitoring (MRM), with precursor-to-fragment ion transitions from MS/MS spectra. Mass analysis was conducted in both ion modes using MRM, with precursor-to-fragment ion transitions from MS/MS spectra. Collision energies were optimized for fragmentation and product ion transmission. The MS operational parameters were as follows: drying gas (N2) flow 15 L/min, nebulizing gas (N_2) 11 L/min, capillary voltage 4.000 V, gas temperature 350°C, heat block 400°C, and interface 350°C. Data were acquired and processed using Agilent MassHunter Software. The method was validated by determining the limit of detection (LOD), limit of quantification (LOQ), and linearity range. Analytical method validation parameters are presented in Table S1, which includes 39 phenolic acids and flavonoids. For each analyte, retention time (RT), LOD, LOQ, and coefficient of determination (R^2) were determined. The high R^2 values (>0.99) demonstrate excellent linearity of the calibration curves, thereby confirming the reliability and accuracy of the LC-ESI-MS/MS quantification. In addition, representative MRM chromatograms of authentic standards are provided in Figure 1, further supporting the reliable identification of major phenolic compounds. Before analysis, 50 mg of the extract was dissolved in 1 mL of methanol and combined with 1 mL of n-hexane. After vortexing at 4°C for 2 min and centrifugation at 9.000 rpm for 10 min, the upper phase was discarded. The methanol phase was diluted 1:9 with distilled water, filtered with a 0.45 μm syringe filter, and injected using an autosampler.

2.5 | Antibacterial Activity

The antibacterial activity of the EO and extracts was determined using the disc diffusion method (Akman et al. 2024a) and minimum inhibitory concentration (MIC) (Akman et al. 2024b) assays. Eight bacterial strains were used: five Gram-positive [*Listeria monocytogenes* (ATCC 51774), *Staphylococcus aureus* (ATCC 6538), *Bacillus cereus* (ATCC 10876), *Clostridium perfringens* (ATCC 13124), *Enterococcus faecium* (ATCC 8459)] and three Gram-negative [*Pseudomonas aeruginosa* (ATCC 15442), *Escherichia coli* (ATCC 25922), *Salmonella enteritidis* (ATCC 13076)]. For disc diffusion, 100 μL of each bacterial suspension (10^8 CFU/mL) was inoculated onto PCA plates. Samples dissolved in 10% DMSO (5 μL) were placed on sterile discs. An imipenem (10 μg disc)

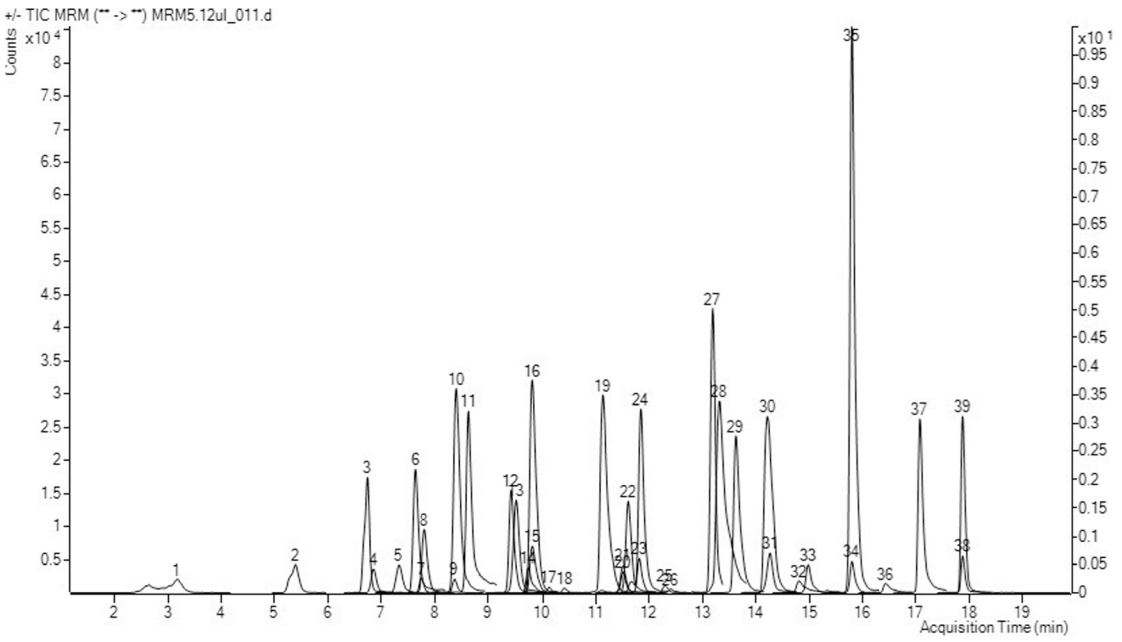

FIGURE 1 | LC-MS/MS Total Ion Chromatogram (TIC) of the standard phytochemicals present in the method.

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was used as the positive control antibiotic. Plates were incubated at 37°C for 24 h, and inhibition zones were measured with a digital caliper.

MIC values were determined using a resazurin-based 96-well microdilution method with slight modifications. 50 µL of sterile Nutrient Broth was added to wells in rows two to ten. 100 µL of the test sample was added to row one, followed by twofold serial dilutions (final concentrations: 1000–1.95µg/mL). Row eleven received 30 µL broth and 30 µL tetracycline (1000 µg/mL) as the positive control antibiotic, while row twelve received 30 µL broth and 30 µL 10% DMSO as the negative control. Then, 10 µL of bacterial suspension (1×10^8CFU/mL) and 30 µL of 0.02% resazurin were added to each well. Plates were incubated at 37°C for 6 h. MIC was recorded as the lowest concentration with no visible color change, indicating inhibition of bacterial growth. All antibacterial assays were performed in triplicate ($n = 3$).

2.6 | Antifungal Activity

The antifungal activity of the EO and extracts was tested against five pathogenic fungi: *Aspergillus flavus* (ATCC 9643), *Fusarium oxysporum* (ATCC 48112), *Alternaria alternata* (ATCC 66981), *Candida albicans* (ATCC 10231), and *Aspergillus niger* (ATCC 16404). The disc diffusion assay was performed following a standard protocol with slight modifications (Bayar et al. 2023). 100 µL of each fungal suspension was inoculated onto Potato Dextrose Agar (PDA), and 10 µL of sample-impregnated discs were placed on the medium. A standard fluconazole (25 mcg disc) was used as a positive control. Petri dishes were incubated for 7 days at 25°C, and inhibition zone diameters were measured using a digital caliper after this period. The experiment was performed in triplicate ($n = 3$).

2.7 | Antioxidant Activity

2.7.1 | DPPH Free Radical Scavenging Activity

The DPPH$^{\bullet}$ radical scavenging capacity of the extracts was determined following a previously described procedure (Akman et al. 2024b; Gözcü et al. 2024). Briefly, 3 mL solutions containing various concentrations (5–100 µg/mL) of test material were mixed with 1 mL of DPPH$^{\bullet}$ solution (0.26 mM). The mixtures were vortexed and incubated at RT for 30 min in the dark. The absorbance was measured at 517 nm. Radical scavenging activity was calculated as: RSA (%) = $[(A_0 - A_1)/A_0] \times 100$, where A_0 is the absorbance of the control reaction (without the test sample), and A_1 is the absorbance in the presence of the extracts or standards. The absorbance values were converted to % activity, and the IC_{50} (µg/mL) concentrations of the extracts were calculated. The results were compared with Trolox, butylated hydroxytoluene (BHT), butylated hydroxy anisole (BHA), and ascorbic acid as a standard antioxidant.

2.7.2 | FRAP

The method described by Albayrak et al. and Şimşek et al. was used to determine the FRAP of the extracts (Albayrak et al. 2024; Şimşek et al. 2025). Stock solutions of extracts (1 mg/mL) were prepared, and 250 µL was mixed with phosphate buffer (0.2 M, pH 6.6) to a total volume of 1.25 mL. Then, 1.25 mL of $K_3Fe(CN)_6$ (1%) was added, and the mixture was incubated at 50°C for 20 min. After incubation, 1.25 mL of TCA (10%) solution and 0.25 mL of $FeCl_3$ (0.1%) solution were added to the reaction mixture. The absorbance of the final mixture was measured at 700 nm. The results were reported as mg Trolox equivalent (TE) activity/g extract, using a calibration curve at different Trolox concentrations (10–100 µg/mL). The results were compared with Trolox, BHT, BHA, and ascorbic acid as standard antioxidants. All analyses were conducted in triplicate, and the results are presented as mean ± standard deviation (SD).

2.7.3 | Total Phenolic Content

The total phenolic content of the extracts was determined spectrophotometrically using the Folin–Ciocalteu reagent (Varol et al. 2023; Gözcü et al. 2025). Briefly, 100 µL of extract and standard solutions (1 mg/mL) were diluted with 4.5 mL of distilled water. The mixtures were combined with 100 µL of Folin–Ciocalteu reagent, followed by the addition of 300 µL of a 2% Na_2CO_3. The final mixtures were vortexed and incubated at room temperature for 120 min. The absorbances were recorded using a spectrophotometer set at 760 nm. The calibration curve ($y =$ 0.117x-0.011) was generated using various concentrations of gallic acid (1–1000 µg/mL). The results were expressed as mg gallic acid equivalent phenolic component (GAE) per gram of extract.

2.7.4 | Total Flavonoid Content

The total flavonoid content of the extracts was determined following a previously described procedure (Akşit et al. 2022; Adıgüzelli et al. 2024). Briefly, 100 µL of extract and standard solutions (1 mg/mL) were mixed with 4.7 mL of methanol. After adding 100 µL of NH_4CH_3COO (1 M) solution and 100 µL of $AlCl_3$ (10%), the mixtures were vortexed and incubated for 45 min at room temperature. After the incubation, the absorbances were measured at 415 nm. The results were reported as mg quercetin equivalent (QE) per gram extract, using a calibration curve drawn with varied quercetin concentrations (1–1000 µg/mL).

2.8 | HPLC-DAD Quantification of RA

Quantitative analysis of RA was conducted using an HPLC-DAD system (Shimadzu) equipped with a Chrometsil C18 column (4.6×150 mm, 5 µm). The method was adapted from a previously reported procedure (Ulutaş et al. 2025) and modified for the analysis of RA. The mobile phase consisted of deionized water containing 2.5% formic acid (A) and methanol (B). The flow rate was set at 1 mL/min. An isocratic flow of 95:5 (A:B) was first applied for 2 min, after which a linear gradient was performed to reach 5:95 (A:B) over 20 min. This was followed by an isocratic phase at 0:100 (A:B) for 3 min, and finally, the conditions were returned to 95:5 (A:B) within 3 min to allow for column equilibration. During the separation, the column temperature was set at 35°C, and detection was carried out at 330 nm. Standard solutions of RA at concentrations of 5, 10, 25, 50, 100, and 200 µg/mL were used to construct a six-point calibration curve ($y =$ 44921x-28888 $R^2 =$ 0.9996). For analysis, extracts were prepared in deionized water at a concentration of 5 mg/mL, filtered through a 0.22 µm syringe filter before injection into the system, and transferred to vials. RA content was expressed relative to the dry weight of each fraction (mg/g extract). RA was quantified using HPLC-DAD instead of LC-MS/MS, as this method provided higher sensitivity, stable

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calibration performance, and more reliable quantification for this compound under the experimental conditions. In addition, RA was identified by comparing its RT and UV–vis absorption spectrum ($\lambda_{max} \approx 320–330$ nm) with those of the authentic standard, and its quantification was validated using a calibration curve constructed with the same standard ($R^2 > 0.999$). Accordingly, the variations observed among fractions can be attributed to the selective affinity of the compound and the compositional concentration of the extracts.

2.9 | Statical Analyses

Statistical analyses were performed using SPSS version 20.0 (IBM Corp., Armonk, NY, USA). One-way ANOVA was applied to determine differences among data, and Duncan's multiple range test was used for post-hoc comparisons at the $p < 0.05$ significance level. Pearson correlation analysis was conducted using two-tailed tests to assess linear relationships between phytochemical parameters and biological activities. Correlation coefficients (r) and their significance levels (p) were reported. All data were expressed as mean ± SD from three independent replicates ($n = 3$).

3 | Results and Discussion

3.1 | Chemical Composition of EO

The EO extracted from SH was analyzed by GC–MS. Identified compounds with their RTs, indices, and percentages are listed in Table 1 and Figure 2. In total, 34 compounds (98.76% of the EO) were identified. The main groups were oxygenated monoterpenes (60.23%), monoterpene hydrocarbons (32.65%), sesquiterpene hydrocarbons (1.42%), oxygenated sesquiterpenes (0.80%), and minor others (0.66%). Oxygenated monoterpenes dominated, consistent with previous studies on *Satureja*, particularly SH, known for high thymol and carvacrol content.

The major EO components were thymol (40.83%), carvacrol (19.77%), γ-terpinene (16.71%), and p-cymene (12.06%), representing over 89% of the composition. Thymol and carvacrol, structurally similar phenolic monoterpenes, exhibit antimicrobial, antioxidant, and anti-inflammatory effects (El-Sayed et al. 2016). Their high concentration highlights potential applications in pharmaceuticals, cosmetics, and food preservation (Alexa et al. 2018). γ-Terpinene and p-cymene are important as biosynthetic precursors of thymol and carvacrol, suggesting an active conversion process in SH, consistent with other *Satureja* species (Bezić et al. 2009). Minor compounds included α-pinene (1.42%), α-terpinolene (1.31%), spathulenol (0.46%), and caryophyllene oxide (0.29%), which, even at low concentrations, contribute significantly to bioactivity and aroma (Xiao et al. 2016). The low sesquiterpene content in Erzincan-grown SH matches temperate region findings but contrasts with Mediterranean reports of higher sesquiterpenes (Mihajilov-Krstev et al. 2009; Hassanzadeh-Khayyat et al. 2012).

Comparative analysis of *Satureja* species shows significant compositional variations. *S. montana* EO has a higher carvacrol-to-thymol ratio (Skočibušić and Bezić 2004), while thymol is the major in this study. Previous reports showed carvacrol as high as 67% (Alexa et al. 2018), and SH from Iran also exhibited higher carvacrol (up to 45%) with lower thymol (Sefidkon and Jamzad 2005). In contrast, *S. laxiflora* (63.9% thymol), *S. montana*, and *S. thymbra* contained high thymol (Chorianopoulos et al. 2006; Pirbalouti et al. 2011), supporting thymol as a genus-specific trait consistent with our findings. These variations may result from genetic, environmental, and geographical factors.

3.2 | Phytochemical Investigation of the Extracts by LC-ESI-MS/MS

The phytochemical profiling of the SH extracts was performed using a previously developed and validated LC-ESI-MS/MS method. This analytical technique analyzed the extracts for their bioactive phytochemical content, quantifying 40 phenolic compounds. Table 2 presents a quantitative analysis of these extracts, while Figure 3 displays the LC-ESI-MS/MS TIC chromatograms of the SH extracts.

The LC-ESI-MS/MS analysis of extracts showed significant variations in bioactive compound profiles and concentrations among the four extraction fractions: WE, ButOH, EtOAc, and WF. This highlights the importance of solvent selection in optimizing phenolic content. The EtOAc was found to have the highest phenolic content, with major compounds including hesperidin (95.42 mg/g), rutin (32.22 mg/g), caffeic acid (5.78 mg/g), sinapic acid (2.88 mg/g), and isoquercitrin (3.24 mg/g). Statistical analysis confirmed that these differences were significant ($p < 0.05$), and the concentrations of the compounds differed significantly among the extracts. These compounds are well-known for their potent antioxidant, anti-inflammatory, antiapoptotic, antidiabetic, antiproliferative, cardioprotective effects (Afanas'eva et al. 2001; Parhiz et al. 2015; Choi et al. 2018). The ButOH fraction also demonstrated a high phenolic content, with considerable amounts of hesperidin (61.26 mg/g), rutin (30.53 mg/g), and scutellarin (1.22 mg/g). Scutellarin, specific to this fraction, demonstrates neuroprotective and anti-ischemic effects, enhancing brain health and reducing oxidative stress in neural tissues(Wang and Ma 2018).

The WE showed lower concentrations of phenolic compounds but contained detectable levels of hesperidin (3.97 mg/g), rutin (1.306 mg/g), and caffeic acid (0.06 mg/g). Although present at lower concentrations, these compounds still showed statistically significant differences ($p < 0.05$), indicating that the WE may contribute to the pharmacological potential of SH. This indicates that while water may not extract high amounts of phenolics, it can extract hydrophilic compounds with bioactive potential. The WF had the lowest bioactive content, suggesting limited capacity for phenolic recovery. However, this fraction may still be significant in isolating specific hydrophilic metabolites or in combination with other extracts for functional applications. These findings suggest that although water is not optimal for extracting high levels of total phenolics from SH, it enables the selective extraction of hydrophilic phenolic compounds like hesperidin, rutin, and caffeic acid. These compounds, even at low concentrations, indicate that the aqueous extract may contribute to pharmacological potential. Water-based extraction is in accordance with the principles of green chemistry and offers significant advantages in the development of formulations for food, cosmetic, and pharmaceutical applications, particularly due to concerns over solvent toxicity (Chemat et al. 2019). The variable profiles among the fractions highlight the critical importance of using multisolvent extraction strategies to enable the comprehensive extraction of bioactive compounds from SH.

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TABLE 1 | The chemical composition of SH EO.

Comp. No.	RT	RI*	RI**	Component	% Composition***	Comp. No.	RT	RI*	RI**	Component	% Composition***
1	3.87	842	847	2-hexenal	0.05 ± 0.01	18	12.53	1185	1187	4-terpineol	0.69 ± 0.18
2	5.53	911	906	Thujene	0.20 ± 0.03	19	12.74	1193	1189	*p*-cymene-8-ol	0.06 ± 0.01
3	6.83	913	917	α-pinene	1.42 ± 0.12	20	14.37	1240	1245	Carvacrol methyl ether	0.30 ± 0.04
4	7.19	980	986	α-myrcene	0.83 ± 0.06	21	15.77	1299	1296	Thymol	**40.83 ± 1.10**
5	7.31	989	993	3-octanol	0.09 ± 0.02	22	16.01	1313	1317	Carvacrol	**19.77 ± 0.22**
6	7.56	996	1000	α-phellandrene	0.14 ± 0.02	23	17.42	1348	1353	Eugenol	0.07 ± 0.02
7	7.73	1005	1007	3-carene	0.04 ± 0.01	24	17.81	1389	1391	Carvacryl acetate	0.11 ± 0.02
8	8.14	1018	1021	*p*-cymene	**12.06 ± 0.60**	25	19.08	1467	1465	Caryophyllene	0.62 ± 0.10
9	8.25	1025	1027	Limonene	0.46 ± 0.04	26	19.56	1480	1484	Aromadendrene	0.09 ± 0.01
10	8.50	1037	1041	Cis-ocimene	0.19 ± 0.03	27	19.93	1483	1488	α-humulene	0.04 ± 0.00
11	8.80	1048	1050	Trans-β-ocimene	0.29 ± 0.05	28	20.60	1491	1496	Germacrene D	0.22 ± 0.03
12	9.13	1054	1058	γ-terpinene	**16.71 ± 0.06**	29	20.98	1503	1499	Bicyclogermacrene	0.35 ± 0.02
13	9.36	1064	1067	*Cis*-sabinene hydrate	0.12 ± 0.02	30	21.22	1535	1540	α-bisabolene	0.10 ± 0.01
14	9.97	1084	1088	α-terpinolene	1.31 ± 0.10	31	22.90	1553	1557	Spathulenol	0.46 ± 0.05
15	10.28	1094	1098	Linalool	0.67 ± 0.04	32	23.04	1580	1583	Caryophyllene oxide	0.29 ± 0.06
16	10.92	1173	1179	α-Terpineol	0.07 ± 0.01	33	24.55	1650	1652	α-eudesmol	0.05 ± 0.00
17	11.43	1180	1182	Isopinocarveol	0.14 ± 0.02	34	28.55	1682	1687	Nerolidol-epoxy acetate	0.04 ± 0.01

Class of compounds	
Monoterpenes (2, 3, 4, 6, 7, 8, 9, 10, 11, 12, and 14)	32.65
Oxygenated monoterpenes (15, 16, 17, 18, 19, 21, and 22)	60.23
Sesquiterpenes (25, 26, 27, 28, 29, and 30)	1.42
Oxygenated sesquiterpenes (31, 32, and 33)	0.80
Others (1, 5, 20, 23, 24, and 34)	0.66
Total identified	98.76

Note: *Retention index determined on an HP-5 MS column using the homologous series of n-hydrocarbons. **Retention index values obtained from NIST webbook. ***Major compounds (>5%) highlighted in bold.

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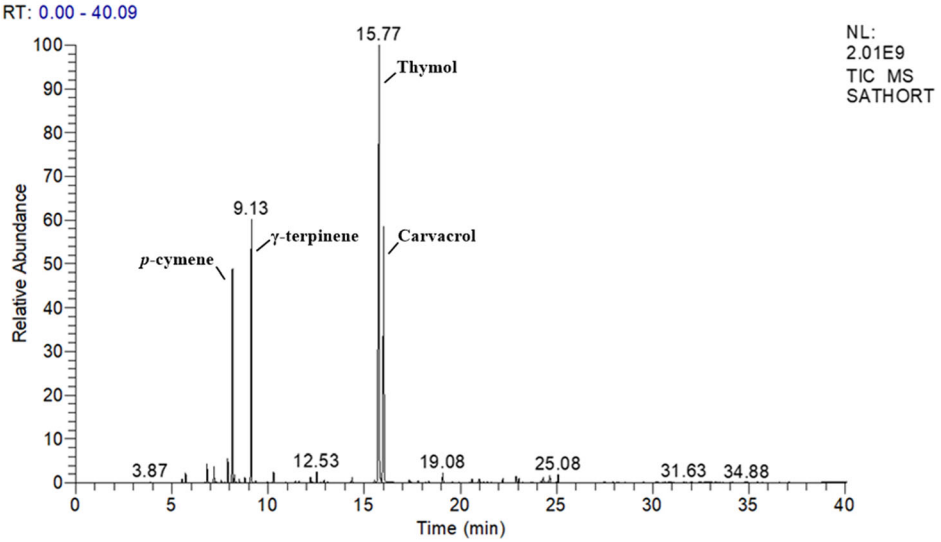

FIGURE 2 | GC/MS chromatogram of the EO of SH.

TABLE 2 | LC-ESI-MS/MS quantitative results of the solvent-based extracts of SH (mg analyte/g extract).

No	[a]Compound Name	RT	EtOAc	ButOH	WE	WF
2	Protocatechuic acid	6.024	1.27 ± 0.18b	0.15 ± 0.03a	ND	ND
5	Chlorogenic acid	7.396	0.09 ± 0.01a	0.10 ± 0.01a	ND	ND
6	Hydroxybenzaldeyde	7.634	0.13 ± 0.01a	0.01 ± 0.01a	ND	ND
7	Vanillic acid	7.688	1.19 ± 0.09b	ND	ND	ND
8	Caffeic acid	7.937	5.78 ± 0.12c	0.43 ± 0.03b	0.06 ± 0.01a	ND
9	Syringic acid	8.437	4.82 ± 0.10c	0.34 ± 0.02b	ND	ND
11	Vanillin	8.756	0.48 ± 0.15ab	0.01 ± 0.01a	ND	ND
12	o-coumaric acid	9.456	1.00 ± 0.08b	0.04 ± 0.02a	0.01 ± 0.01a	ND
13	Salicylic acid	9.566	0.79 ± 0.02b	0.09 ± 0.02a	0.02 ± 0.01a	ND
14	Taxifolin	9.756	0.07 ± 0.02a	ND	ND	ND
15	Resveratrol	9.733	ND	0.01 ± 0.01a	ND	ND
16	Polydatin	9.827	ND	0.01 ± 0.01a	ND	ND
17	trans-ferulic acid	10.190	3.37 ± 0.09c	0.11 ± 0.05a	ND	ND
18	Sinapic acid	10.346	2.88 ± 0.08c	0.12 ± 0.01a	ND	ND
19	Scutellarin	10.593	0.87 ± 0.04b	1.22 ± 0.17b	0.09 ± 0.02a	0.04 ± 0.01a
23	Hesperidin	11.836	95.42 ± 1.66e	61.26 ± 1.70d	3.97 ± 0.5c	0.87 ± 0.04b
24	Isoquercitrin	11.853	3.24 ± 0.12c	0.76 ± 0.05b	0.03 ± 0.01a	ND
25	Rutin	11.976	32.22 ± 1.56d	30.53 ± 1.88c	1.31 ± 0.09b	0.29 ± 0.03a
27	Kaempferol-3-glucoside	13.207	0.12 ± 0.03a	ND	ND	ND
31	trans-cinnamic acid	14.249	0.05 ± 0.01a	ND	ND	ND
32	Naringenin	14.990	1.7 ± 0.17b	0.05 ± 0.01a	ND	ND

Note: Different letters (a–e) in the same column indicate statistical difference ($p < 0.05$).
Abbreviations: BuOH, n-Butanol fraction; EtOAc, Ethyl acetate fraction; N.D., not detected; WE, water extract; WF, water fraction.
[a]Gallic acid, epigallocatechin, catechin, caffeine, *p*-coumaric acid, coumarin, protocatechuic ethyl ester, quercetin-3-xyloside, fisetin, baicalin, chrysin, quercetin, hesperetin, morin, kaempferol, baicalein, luteolin, and biochanin A were analyzed but not detected in any of the samples. Values are expressed as mean ± SD ($n = 3$).

3.3 | Antioxidant Activity of Extracts

The antioxidant potential of SH extracts was determined by DPPH• scavenging activity, TPC, TFC, and FRAP. The findings are presented in Table 3. The DPPH• scavenging activity of the extracts was quantitatively evaluated and expressed as IC_{50} values (μg/mL). The EtOAc demonstrated the highest radical scavenging activity with an IC_{50} value of 17.25 ± 2.34 μg/mL, followed by the BuOH (25.70 ± 2.02 μg/mL) and the WE (75.07±1.46 μg/mL). The WF exhibited the lowest activity with an IC_{50} value of 135.70 ± 1.18 μg/mL. Compared to synthetic antioxidants such as BHA

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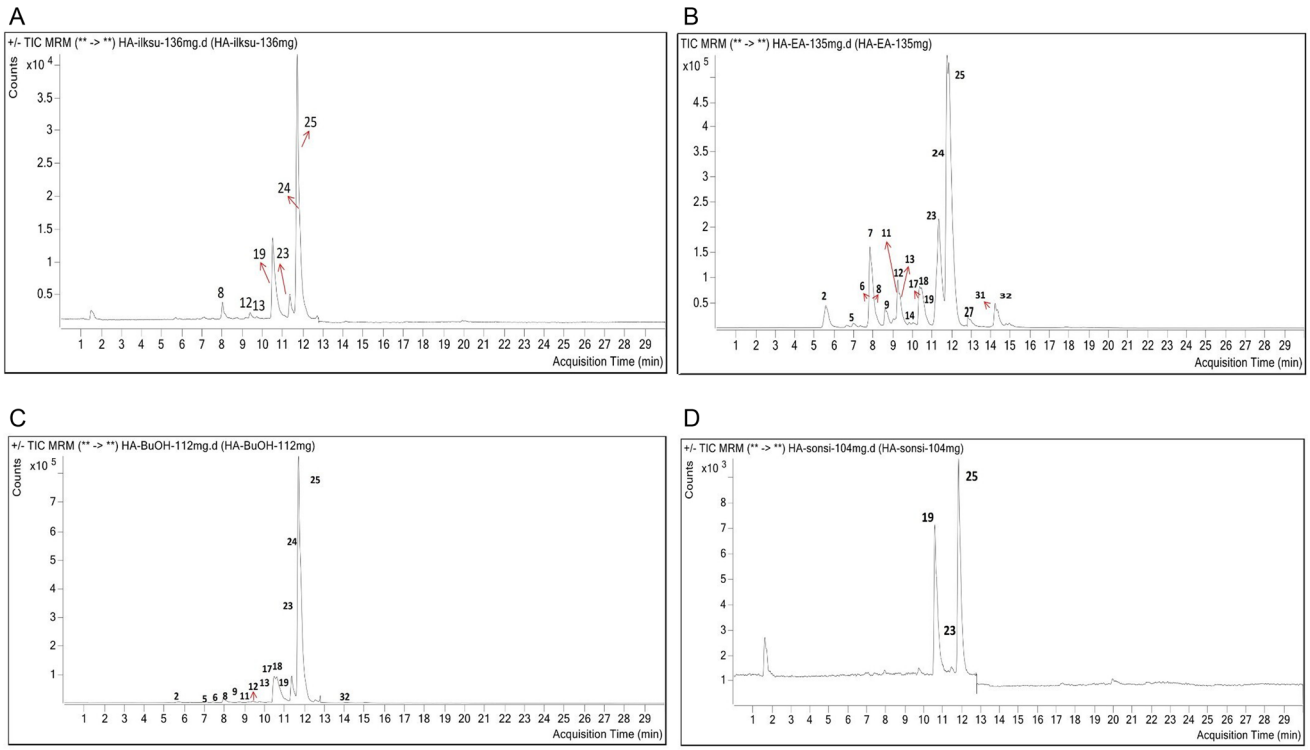

FIGURE 3 | TICs of the (A) WE, (B) EtOAc fraction, (C) BuOH fraction (D) WF.

TABLE 3 | Total phenolic, flavonoid contents, and antioxidant activity of SH.

Sample	DPPH• scavenging IC_{50}, μg/mL	Total phenolic, mg GAE/g extract	Total flavonoids, mg QE/g extract	Reducing power, mg TE/g extract
WE	75.07 ± 1.46d	27.47 ± 1.44b	48.21 ± 1.56c	34.18 ± 0.44b
EtOAc	17.25 ± 2.34b	79.24 ± 0.59d	19.14 ± 0.38a	96.86 ± 1.06d
ButOH	25.70 ± 2.02c	55.76 ± 0.72c	37.48 ± 0.22b	64.23 ± 0.55c
WF	135.70 ± 1.18e	19.58 ± 0.59a	14.90 ± 0.54a	18.14 ± 1.08a
Trolox	8.50 ± 0.77a	—	—	—
BHA	8.04 ± 0.69a	—	—	338.57 ± 0.31f
BHT	10.70 ± 0.73ab	—	—	257.80 ± 1.24e
Ascorbic acid	9.91 ± 0.87a	—	—	383.66 ± 0.98g

Note: Different letters (a–g) in the same column indicate statistical difference ($p < 0.05$). Values are expressed as mean ± SD ($n = 3$).
Abbreviations: BuOH, n-Butanol fraction; EtOAc, Ethyl acetate fraction; WE, water extract; WF, water fraction.

(8.04 ± 0.69 μg/mL), BHT (10.70 ± 0.73 μg/mL), ascorbic acid (9.91 ± 0.87 μg/mL), and Trolox (8.50 ± 0.77 μg/mL), the extracts demonstrated moderate to strong antioxidant potential, with the EtOAc showing particularly significant antioxidant activity. Statistical analysis revealed that the IC_{50} values differed significantly among all samples ($p < 0.05$), indicating that the DPPH• scavenging activities of the extracts were statistically distinct. These results support previous findings on *Satureja* species (Abdelshafeek et al. 2023). The lower IC_{50} values observed for the EtOAc and BuOH fractions support the idea that fractions characterized by high levels of phenolics and flavonoids significantly contribute to free radical scavenging activity (Herlina et al. 2018).

The TPC was highest in the EtOAc (79.24 ± 0.59 mg GAE/g), followed by BuOH (55.76 ± 0.72 mg GAE/g) and the WE (27.47 ± 1.44 mg GAE/g), with the lowest in the WF (19.58 ± 0.59 mg GAE/g). These findings indicate that EtOAc contains the highest level of phenolic compounds, which may be attributed to its strong antioxidant activity. In TFC analysis, the WE had the highest flavonoid level (48.21 ± 1.56 mg QE/g), followed by BuOH (37.48 ± 0.22 mg QE/g) and EtOAc (19.14 ± 0.38 mg QE/g), while the lowest was again in the WF (14.90 ± 0.54 mg QE/g). The statistical groupings showed that the WE and BuOH differed significantly from EtOAc and WF ($p < 0.05$), highlighting the higher flavonoid solubility in water-based extracts. The higher flavonoid content in the WE suggests that flavonoids in SH may be more soluble in aqueous solvents compared to polyphenolic compounds, which are typically found in organic solvents such as ethyl acetate and butanol (Fikayuniar et al. 2023). The strong correlation between phenolic content and antioxidant capacity supports the hypothesis that phenolics

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are major contributors to the radical scavenging activity of *Satureja* species (Al-Assaf and Khazem 2021).

The reducing power of the extracts, expressed as Trolox equivalents, was highest in the EtOAc (96.86 ± 1.06 mg TE/g), followed by BuOH (64.23 ± 0.55 mg TE/g) and the aqueous extract (34.18 ± 0.44 mg TE/g), with the lowest in the aqueous fraction (18.14 ± 1.08 mg TE/g). Synthetic antioxidants such as ascorbic acid (383.66 ± 0.98 mg TE/g), BHA (338.57 ± 0.31 mg TE/g), and BHT (257.80 ± 1.24 mg TE/g) showed significantly higher reducing capacities. These values were significantly different across all samples ($p < 0.05$), with each fraction classified into a separate significance group, confirming the solvent-dependent variation in electron-donating capacity. These findings are in agreement with previous studies indicating that *Satureja* species demonstrate strong reducing capacity, particularly in organic solvent fractions, further supporting the view that fractions characterized by high phenolic content are more potent electron donors (Babajafari et al. 2015). These results provide important information on the solvent-dependent extraction of bioactive compounds from SH, highlighting its potential as a natural antioxidant source.

3.4 | Antibacterial Activity of EO and Extracts

The antibacterial activity of EO and extracts was determined against five Gram-positive and three Gram-negative bacterial strains using disc diffusion and MIC techniques. The results, presented in Table 4, indicate that the EO demonstrated significant antibacterial activity, particularly against Gram-positive bacteria. The most significant inhibitory effects were observed against *S. aureus* (45 mm), *B. cereus* (31 mm), and *L. monocytogenes* (31 mm). The inhibition zones of the EO against these three Gram-positive strains were statistically higher than those observed for all solvent-based extracts ($p < 0.05$), confirming its superior antibacterial activity. Notably, the zone of inhibition for *S. aureus* was not significantly different from that produced by the positive control antibiotic imipenem (40 mm), indicating comparable potency against this bacterial strain. The significant inhibition observed against *S. aureus* is particularly important, as this pathogen is recognized as a critical antibiotic-resistant bacterium, including methicillin-resistant *S. aureus*, a major cause of hospital-acquired infections (Chambers and DeLeo, 2009). The inhibition zones observed for these bacteria suggest that EO exhibits strong bactericidal properties, which may be attributed to its ability to compromise bacterial membrane integrity and interfere with intracellular metabolic processes, resulting in cell death (Cherrat et al. 2016; Bordón et al. 2025).

The EO also showed significant antibacterial activity against Gram-negative bacteria, particularly *E. coli* (32 mm), *S. enteritidis* (30 mm), and *P. aeruginosa* (22 mm). Gram-negative bacteria are generally more resistant to antimicrobial agents due to an outer membrane composed of lipopolysaccharides, which serves as a selective permeability barrier (Abdi et al. 2021). Despite this, the results indicate that EO was still effective against these bacterial species, suggesting that it exhibits wide-ranging antimicrobial properties. The inhibition zones for *E. coli* and *S. enteritidis* were not significantly different from those produced by the positive control antibiotic imipenem ($p > 0.05$), further supporting the comparable antibacterial activity of the EO when tested against these bacterial strains. However, the inhibition zone observed for *P. aeruginosa* was significantly lower than that of imipenem ($p < 0.05$), though still statistically higher than all extracts tested ($p < 0.05$), indicating partial susceptibility. The inhibition zones for *E. coli* and *S. enteritidis* were similar to those of imipenem, used as a positive control. The observed antibacterial activity against Gram-negative bacteria may be attributed to a combination of cell membrane disruption, inhibition of ATP synthesis, and induction of oxidative stress as a result of reactive oxygen species generation, which collectively result in bacterial cell death (Wang et al. 2013). Among tested bacterial strains, *P. aeruginosa* showed the highest resistance, consistent with its intrinsic multidrug resistance mechanisms like efflux pumps and low membrane permeability (Kello et al. 2023). However, the fact that EO demonstrated an inhibitory effect against *P. aeruginosa* suggests that it may be a promising candidate for combination therapies with traditional antimicrobial agents. The strong antibacterial activity may be attributed to the high thymol (40.83%) and carvacrol (19.77%) content, known for their membrane-disrupting properties, resulting in increased permeability and cell lysis (Güllüce et al. 2003).

Solvent-based SH extracts, EtOAc, BuOH, water, and WF, were tested for antibacterial activity. EtOAc and BuOH fractions showed moderate inhibition against Gram-positive strains such as *S. aureus*, *L. monocytogenes*, and *C. perfringens*, suggesting that phenolic-rich organic extracts contribute to antibacterial effects. Statistical analysis revealed that the inhibition zones of the

TABLE 4 | The *in vitro* results on the antibacterial activity of EO and extracts.

Bacteria	EO	EtOAc	BuOH	WE	WF	Imipenem[a]
L. monocytogenes	31.0 ± 1.2a	11.0 ± 1.2c	3.0 ± 1.2d	ni	ni	20.0 ± 3.0b
S. aureus	45.0 ± 0.3a	17.0 ± 0.7b	8.0 ± 0.6c	2.0 ± 0.4d	ni	40.0 ± 0.7a
B. cereus	31.0 ± 1.5a	13.0 ± 1.5c	ni	ni	ni	25.0 ± 1.4b
C. perfringens	28.0 ± 2.1a	9.0 ± 1.0c	ni	ni	ni	13.0 ± 1.6b
E. faecium	23.0 ± 0.9a	ni	ni	ni	ni	18.0 ± 1.3b
P. aeruginosa	25.0 ± 1.0a	ni	ni	ni	ni	22.0 ± 1.4b
E. coli	32.0 ± 1.8a	ni	ni	ni	ni	28.0 ± 0.6b
S. enteriditis	30.0 ± 1.2a	ni	ni	ni	ni	28.0 ± 0.5a

Note: Different letters (a–d) in the same column indicate statistical difference ($p < 0.05$).
Abbreviations: BuOH, n-Butanol fraction; EtOAc, Ethyl acetate fraction; ni., no inhibition; WE, water extract; WF, water fraction.
[a]Positive control (Imipenem), inhibition zone diameters in mm (mean ± sd, $n = 3$).

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EtOAc were significantly lower than those of the EO and imipenem ($p < 0.05$), yet significantly higher than those of the BuOH and WEs ($p < 0.05$), indicating moderate but distinct activity. No inhibition was observed against *B. cereus* and *E. faecium*, indicating selective antibacterial activity. The EtOAc demonstrated activity against selected strains, which may be attributed to its high concentrations of hesperidin, rutin, and caffeic acid, compounds known for their antimicrobial properties (Kostić et al. 2020). In contrast, the WE showed low antibacterial activity, particularly against *S. aureus* and *C. perfringens*, which may be attributed to its lower total phenolic content and antioxidant potential. However, the WF showed no antibacterial activity against the tested bacterial strains. Although limited, the antibacterial activity observed in the WE may be attributed to the content of hydrophilic phenolics such as hesperidin and rutin (Fratianni et al. 2013). These findings suggest that while the EO demonstrates strong antibacterial activity against multiple bacterial strains, some organic solvent extracts may also contribute to the antimicrobial activity, although with lower or selective activity. These findings suggest that while the EO demonstrates strong antibacterial activity against multiple bacterial strains, some organic solvent extracts may also contribute to the antimicrobial activity, although with lower or selective activity. The results support the hypothesis that both the EO and the solvent extracts with high phenolic content from SH may be potential sources of natural antimicrobial agents (Serrano et al. 2011).

The antimicrobial potential of EO and extracts was evaluated by MIC analyses (Table 5), which provide a more reliable measure of antimicrobial efficacy, as inhibition zones in disc diffusion assays can also be influenced by the diffusion ability and polarity of compounds in the agar medium. The EO showed significant activity, particularly against *S. aureus* (31.25 μg/mL), followed by *L. monocytogenes*, *B. cereus*, and *C. perfringens* (62.5 μg/mL), consistent with previous reports on thymol- and carvacrol-rich oils that disrupt microbial membranes (Cristani et al. 2007). However, consistent with our findings, previous studies have shown that although *Satureja* EOs exhibit significant antimicrobial activity, standard antibiotics demonstrate higher potency (Giweli et al. 2012; Miladinović et al. 2014; Akşit et al. 2024). Higher MICs for *E. faecium* (125 μg/mL) and *P. aeruginosa* (250 μg/mL) may relate to intrinsic resistance mechanisms like efflux pumps and low membrane permeability (Kello et al. 2023), though measurable activity was still observed. Despite this, the EO showed measurable antibacterial activity against tested bacterial strains, indicating potential as a natural antimicrobial agent. The EtOAc exhibited moderate activity against *S. aureus* and *L. monocytogenes* (62.5 and 125 μg/mL, respectively), possibly due to phenolic compounds such as hesperidin, rutin, and caffeic acid, which are known to disrupt microbial membranes and interfere with enzymatic processes (Fratianni et al. 2013). In contrast, the WEs and WF (MIC > 500 μg/mL) displayed minimal or no antibacterial activity, consistent with reports that hydrophilic constituents generally have lower antimicrobial activity than lipophilic phenolic compounds in plant extracts (Bunghez et al. 2015; Maslov et al. 2024). These MIC results emphasize the role of phenolic monoterpenes such as thymol and carvacrol in the antibacterial potency of EO and support its potential use in pharmaceutical, cosmetic, and food-related applications as an alternative to synthetic preservatives and antibiotics.

3.5 | Antifungal Activity of EO and Extracts

The antifungal properties of EO and extracts were evaluated against *A. flavus*, *F. oxysporum*, *A. alternata*, *C. albicans*, and *A. niger* by measuring inhibition zones (in millimeters) and comparing them with fluconazole (Table 6). EO demonstrated significant antifungal activity, with inhibition zones between 9 and 50 mm, based on the fungal species tested. The strongest effect was against *A. niger* (50 mm), followed by *C. albicans* (43 mm), while *A. flavus* showed the lowest inhibition (9 mm). EO showed higher activity than fluconazole used as a standard against all tested fungi, and it has the potential to be an important antifungal agent due to its antifungal properties. Statistical evaluation of the inhibition zone data revealed that the antifungal activity of the EO was significantly greater than that of fluconazole ($p < 0.05$) in all tested fungal strains, except for *A. flavus*, where no statistically significant difference was observed between the two agents ($p > 0.05$). Moreover, distinct statistical groups were identified for each fungus, indicating that EO exhibited superior antifungal activity in comparison to solvent-based extracts ($p < 0.05$).

The strong antifungal activity of EO suggests that its bioactive constituents inhibit fungal growth by disrupting membrane integrity and increasing permeability. The inhibition of *F. oxysporum* (24 mm) is particularly important, as this pathogen is widely reported to be associated with significant agricultural damage (Gaspar et al. 2014). These findings are consistent with previous studies reporting the antifungal activity of EOs characterized by high levels of thymol and carvacrol, which are known to

TABLE 5 | MIC values of SH EO, extracts, and positive control.

Bacteria	EO	EtOAc	BuOH	WE	WF	Tetracycline
L. monocytogenes	62.5	125	250	>500	>500	3.91
S. aureus	31.25	62.5	250	500	>500	1.95
B. cereus	62.5	125	>500	>500	>500	7.81
C. perfringens	62.5	250	>500	>500	>500	1.95
E. faecium	125	>500	>500	>500	>500	1.95
P. aeruginosa	250	>500	>500	>500	>500	7.81
E. coli	62.5	>500	>500	>500	>500	7.81
S. enteriditis	125	>500	>500	>500	>500	3.91

Abbreviations: BuOH, n-Butanol fraction; EtOAc, Ethyl acetate fraction; WE, water extract; WF, water fraction.

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TABLE 6 | The in vitro results on the antifungal activity of EO and extracts.

Fungi	EO[a]	EtOAc	BuOH	WE	WF	Fluconazole[b]
Aspergillus flavus	9 ± 1.0a	ni	ni	ni	ni	7 ± 0.6a
Fusarium oxysporum	24 ± 1.6a	9 ± 1.2c	ni	ni	ni	15 ± 0.9b
Alternaria alternate	29 ± 1.8a	10 ± 1.3c	7 ± 1.1d	ni	ni	18 ± 0.8b
Candida albicans	43 ± 1.2a	15 ± 0.9c	9 ± 1.4d	5 ± 0.5e	ni	25 ± 0.7b
Aspergillus niger	50 ± 0.7a	ni	ni	ni	ni	21 ± 0.6b

Note: Different letters (a–d) in the same column indicate statistical difference ($p < 0.05$).
Abbreviations: BuOH, n-Butanol fraction; EtOAc, Ethyl acetate fraction; ni., no inhibition; WE, water extract; WF, water fraction.
[a]The inhibition zones are shown in mm.
[b]Positive control (Fluconazole).

destabilize fungal membranes and inhibit spore germination (Zhang et al. 2019). Growing resistance to synthetic fungicides has driven interest in plant-based alternatives, including EOs with activity against phytopathogenic fungi (Nehela et al. 2023). The findings of this study provide strong evidence that EO exhibits potent antifungal activity, supporting its potential application as a natural alternative to synthetic fungicides.

The EO exhibited antifungal activity; however, most solvent-based extracts, including EtOAc, BuOH, WE, and aqueous fraction, showed limited effects. The EtOAc inhibited *F. oxysporum* (9 mm), *A. alternata* (10 mm), and *C. albicans* (15 mm); BuOH was active against *A. alternata* (7 mm) and *C. albicans* (9 mm); the WE showed slight activity only against *C. albicans* (5 mm), while the WF showed none. Statistical analysis confirmed that the inhibition zones produced by these extracts were significantly smaller than those of the EO and fluconazole ($p < 0.05$) in all relevant comparisons. In particular, in *C. albicans*, a gradual decrease in antifungal effect was evident, with each extract classified into a separate statistical group ($p < 0.05$). This trend highlights the relevance of lipophilicity and chemical composition to antifungal activity. These results suggest that the phenolic compounds obtained using polar solvents may exhibit limited lipophilicity or volatility, which could reduce their ability to interfere with fungal membrane integrity. This observation is consistent with previous studies showing that plant extracts with high phenolic content generally demonstrate lower antifungal activity compared to EOs (El Khoury et al. 2017; Belmimoun et al. 2020). Thus, while SH extracts possess antioxidant and antibacterial properties, their antifungal potential as single agents appears limited.

3.6 | RA Contents of Extracts

The quantitative determination of RA in extracts was performed using HPLC-DAD analysis, and the results are presented in Table 7. A significant variation in RA content was observed among the tested samples, reflecting the influence of solvent polarity and extract composition on compound solubility and extractability. The EtOAc exhibited the highest RA concentration

TABLE 7 | RA content of extracts.

Sample	mg RA/g extract
WE	73.15± 0.80c
EtOAc	580.93± 1.12a
ButOH	159.53± 1.35b
WF	9.38± 0.45d

Note: Values are expressed as mean ± SD ($n = 3$). Different letters (a–d) in the same column indicate statistical difference ($p < 0.05$).
Abbreviations: BuOH, n-Butanol fraction; EtOAc, Ethyl acetate fraction; WE, water extract; WF, water fraction.

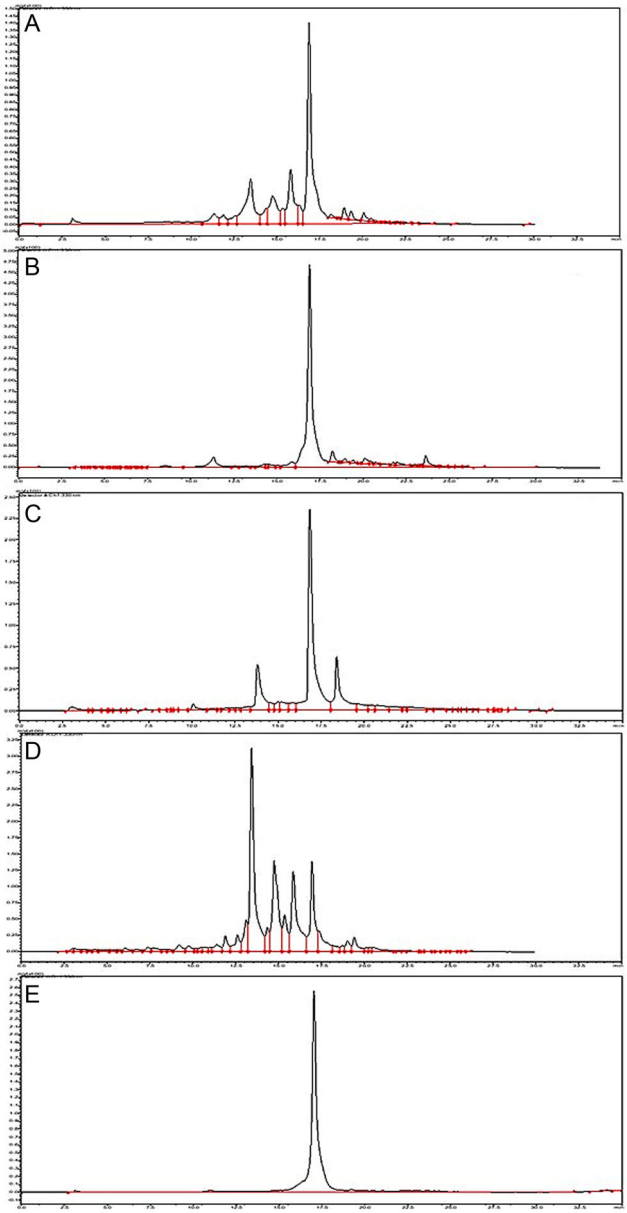

FIGURE 4 | HPLC–DAD chromatograms (330 nm) of (A) WE, (B) EtOAc fraction, (C) BuOH fraction, (D) WF, (E) Standard RA.

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(580.93 ± 1.12 mg/g extract), followed by BuOH (159.53 ± 1.35 mg/g extract) and WE (73.15 ± 0.80 mg/g extract), while the WF contained considerably lower levels (9.38 ± 0.45 mg/g extract). Statistical analysis confirmed that these differences were significant ($p < 0.05$). This distribution profile indicates that RA, a moderate-polarity phenolic acid, is preferentially extracted by semi-polar solvents such as ethyl acetate and n-butanol, consistent with previous phytochemical investigations (Lau and Chua 2019; Guan et al. 2022). The notably high content in EtOAc may be attributed to the optimum polarity for phenolic acid solubilization and reduced interference from highly polar matrix constituents prevalent in aqueous systems. This observation can be explained by the calculation of mg/g extract values relative to the dry weight of each fraction, the enrichment of RA in the ethyl acetate fraction, and its depletion of sugars and other polar constituents. In contrast, the WE, characterized by a high content of polar compounds, has a greater dry weight but a lower relative proportion of RA, resulting in lower concentration values (mg/g extract). Although the WE may contain a higher absolute amount of RA due to its greater total mass, the ethyl acetate fraction exhibits a markedly higher concentration on a per-weight basis, consistent with the high affinity of phenolic acids for solvents of intermediate polarity. The HPLC-DAD chromatograms of the WE, organic fractions, and RA standard (Figure 4) confirmed RA as one of the major phenolic constituents in the analyzed samples. The presence of two catechol moieties in its structure accounts for its strong electron-donating and radical-scavenging properties (Muzolf et al. 2008; Mesa et al. 2012).

The Pearson correlation analysis (Table S2) demonstrated a very strong positive correlation between TPC and FRAP ($r = 0.997$), indicating that phenolic compounds are the principal contributors to the reducing capacity of the extracts. The RA content also exhibited a strong positive correlation with TPC ($r = 0.931$) and FRAP ($r = 0.942$), as shown in Figure 5, confirming its role as a major determinant of redox-based antioxidant mechanisms. These results are consistent with previous reports describing the catechol structure of RA, which enables effective electron donation and transition metal chelation, resulting in an increased ferric-reducing potential (Psotová et al. 2003; David et al. 2020). The negative correlations between DPPH and TPC ($r = -0.901$), FRAP ($r = -0.913$), and RA ($r = -0.743$), as shown in Figure 5, indicate that higher phenolic and RA content is associated with lower IC_{50} values, reflecting stronger radical-scavenging activity. This observation supports previous findings that phenolic hydroxyl groups facilitate both hydrogen atom transfer and single electron transfer mechanisms in radical neutralization (Di Meo et al. 2013; Marković et al. 2019). In contrast, TFC exhibited low correlations with FRAP ($r = -0.128$) and RA ($r = -0.320$), indicating that flavonoids do not constitute the main contributors to the reducing or radical-scavenging capacity in these extracts, possibly due to structural differences in hydroxylation profiles and glycosylation.

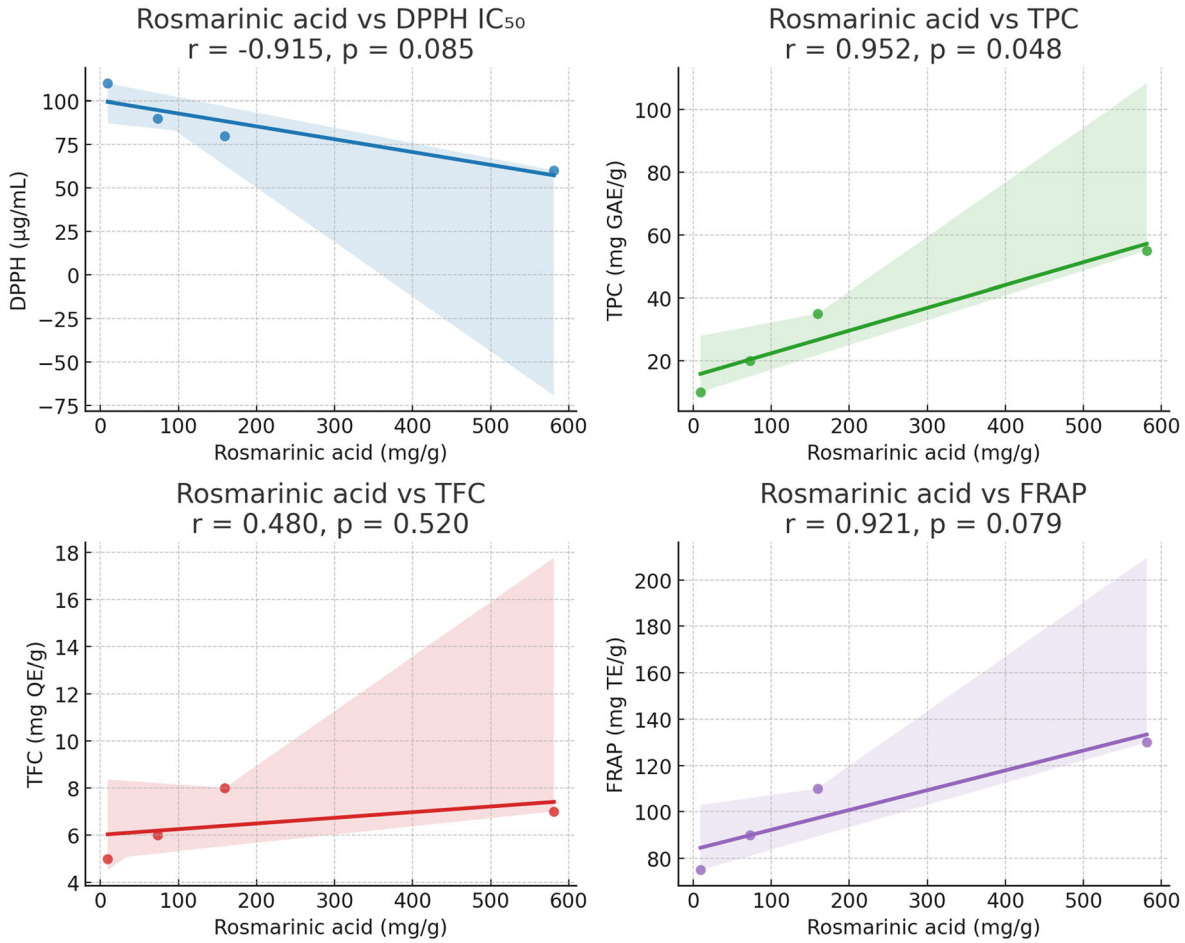

FIGURE 5 | Correlation between RA content and antioxidant activity of extracts.

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The antibacterial activity results (Table S3) revealed strong and significant negative correlations between RA content and MIC values for all tested bacteria. The highest correlations were observed for *B. cereus* and *C. perfringens* ($r = -0.971$), followed by *S. aureus* ($r = -0.928$) and *L. monocytogenes* ($r = -0.887$). These relationships are presented in Figure 6. These results indicate that higher RA concentrations are associated with stronger antibacterial effects. This activity may be explained by multiple mechanisms, including disruption of bacterial cell membrane integrity, inhibition of essential metabolic enzymes, and chelation of metal ions critical for microbial growth (Renzetti et al. 2020; Paterson et al. 2022). The high correlation values suggest that RA plays a significant role in the antibacterial activity of these extracts, although synergistic contributions from other phenolics cannot be disregarded.

The antifungal activity results (Table S4) demonstrated very strong positive correlations between RA content and inhibition of *F. oxysporum* ($r = 0.971$), *C. albicans* ($r = 0.928$), and *A. alternata* ($r = 0.877$), as presented in Figure 7. These data suggest that higher RA concentrations are associated with increased antifungal activity, with the strongest effect observed against *F. oxysporum*. The mechanism responsible for this activity may involve multiple processes, including disruption of fungal cell membrane integrity, ergosterol biosynthesis, chelation of essential metal ions required for enzymatic function, and induction of oxidative stress within fungal cells (Khan et al. 2011; Lyagin et al. 2023).

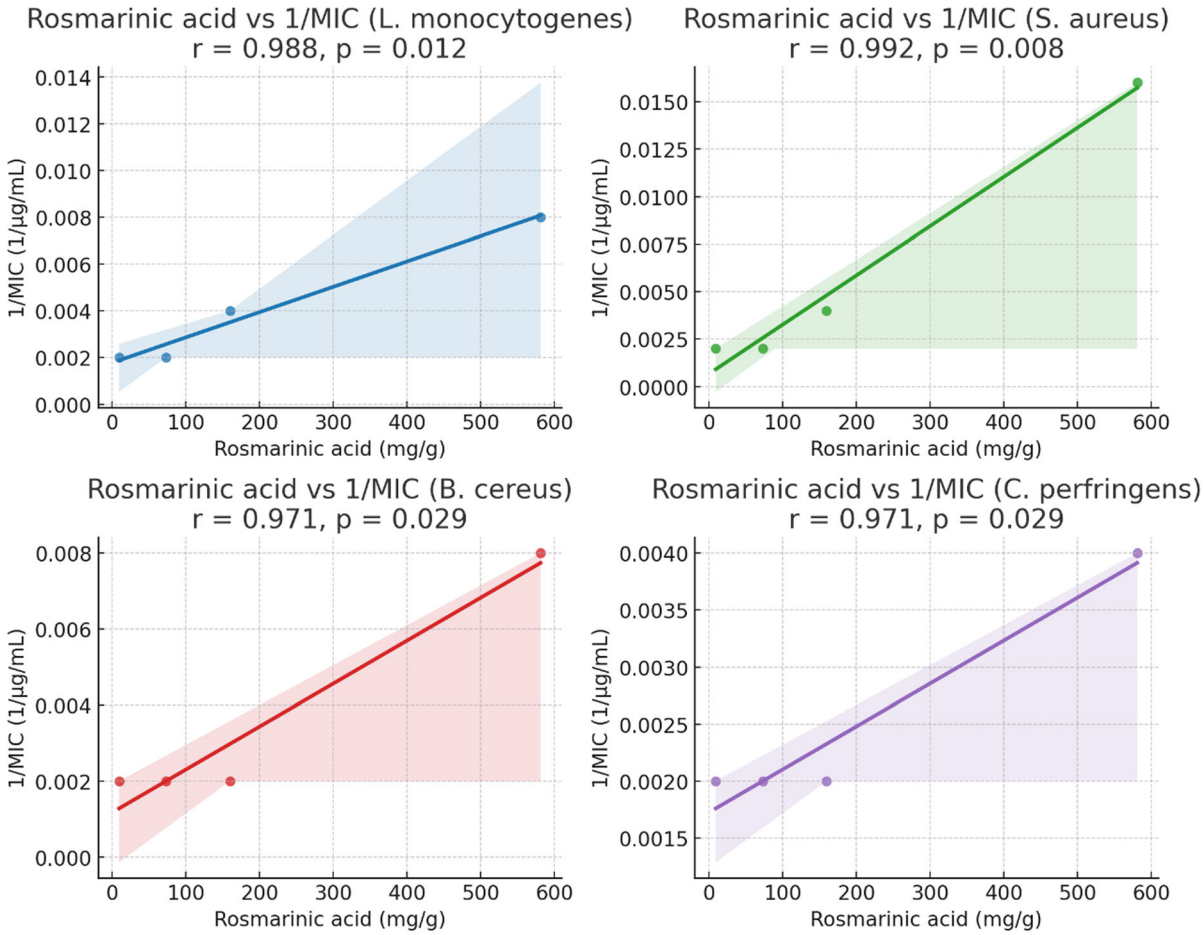

FIGURE 6 | Correlation between RA content and antibacterial activity of extracts.

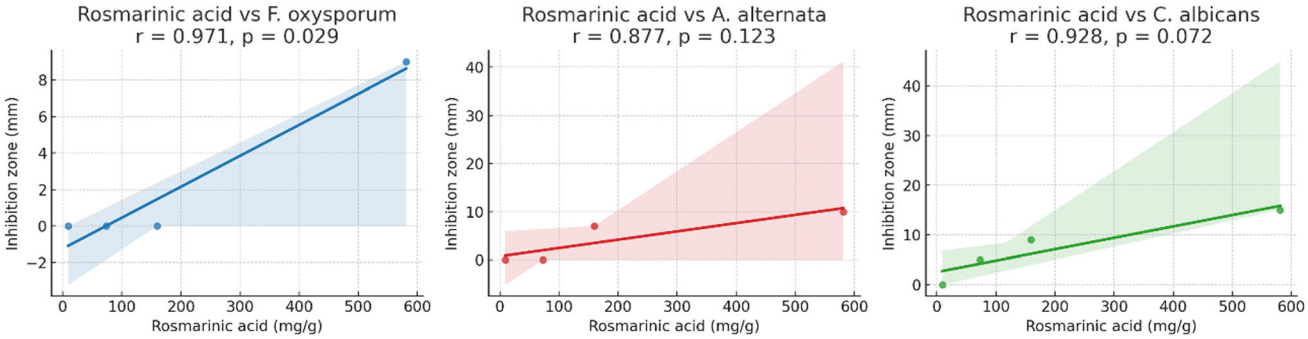

FIGURE 7 | Correlation between RA content and antifungal activity.

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Such multifaceted effects may account for the broad-spectrum antifungal activity observed in the present research. Although the strong correlations indicate a central role for RA, the possible contribution of other phenolic compounds present in the extracts should not be disregarded. Synergistic interactions among these constituents may increase overall antifungal activity, indicating the need for further fractionation and combinatory investigations to determine individual and combined effects. These findings highlight the importance of integrating specific metabolite quantification with comprehensive bioactivity assays to fully elucidate the pharmacological potential of plant-derived compounds.

4 | Conclusion

This study comprehensively investigated the antioxidant, antibacterial, and antifungal activities of extracts and EO, as well as the quantification of major phenolic constituents, including RA, hesperidin, rutin, and caffeic acid. The EtOAc and BuOH fractions exhibited the strongest biological activities, consistent with their high total phenolic and flavonoid contents and elevated concentrations of bioactive compounds. The EtOAc demonstrated the highest RA content and showed significant antioxidant potential, as well as strong inhibition of microbial growth. In contrast, the WE and WF showed minimal activity, correlating with their lower phenolic content and chemical diversity. The EO exhibited potent antibacterial and antifungal activities, attributed to its high levels of thymol and carvacrol. Correlation analyses supported a strong association between phenolic composition, particularly RA, and antioxidant and antimicrobial activities. Overall, these results highlight the pharmacological potential of SH, particularly its EtOAc and BuOH fractions, as sources of natural bioactive compounds. Further investigations involving compound isolation, mechanistic assays, and in vivo evaluations are needed to validate the therapeutic potential of these extracts in the development of functional foods, phytotherapeutics, or complementary interventions for oxidative stress-related and microbial diseases.

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The author has nothing to report.

Conflicts of Interest

The author declares no conflicts of interest.

Data Availability Statement

Data will be made available on request.

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Supporting Information

Additional supporting information can be found online in the Supporting Information section. **Supporting Table S1:** Analytical method validation parameters that belong to the LC-ESI-MS/MS. **Supporting Table S2:** Pearson correlation coefficients between antioxidant activities and rosmarinic acid content. **Supporting Table S3:** Pearson correlation between antibacterial activity (MIC) and rosmarinic acid content. **Supporting Table S4:** Pearson correlation coefficients between antifungal activity and rosmarinic acid content.

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