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Research Article

The Effect of Phenological Stages of *Salvia abrotanoides* (Kar.) Sytsma on Antibacterial and Antioxidant Potential, Total Phenol and Flavonoid Content of Roots and Aerial Parts

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Abstract

This study aimed to evaluate the antibacterial and antioxidant activities of methanol and ethyl acetate extracts of the roots and aerial parts of *Salvia abrotanoides* obtained at different phenological stages (vegetative, flowering, and seeding) and to determine their total phenol and flavonoid content. Disc diffusion and micro-dilution methods evaluated antibacterial activity against eight bacterial strains. Folin-Ciocalteu and aluminum chloride colorimetric methods were used to determine the content of total phenol and flavonoids, respectively. The antioxidant potential of the extracts was measured using a 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay. *Staphylococcus aureus* and *Pseudomonas aeruginosa* were the most sensitive and resistant bacteria to the extracts, respectively. The strongest antibacterial activity against multi-drug resistant bacteria was recorded for methicillin-resistant *Staphylococcus aureus* treated with ethyl acetate extract of the root at the seeding stage, in which MIC and MBC values were 30.33 and 40.00 mg/mL, respectively. The highest content of total phenol (557.51 mg GAE/g DW) and flavonoids (236.40 mg QE/g DW) was found in the ethyl acetate extract of the aerial parts in the seeding phase. The aerial parts had more total phenolic and flavonoid content at different phenological stages than the root. The antioxidant capacity of the aerial part was also better that of the roots. The ethyl acetate extract of the aerial part at the seeding phase presented the highest DPPH scavenging activity (92.51 ± 1.25 %). The results showed that *S. abrotanoides* extracts, especially at the seeding phase, have good potential as a source of antioxidant, antibacterial, and bioactive compounds and can be considered good candidates in the development of new drugs or as the main source of food preservative compounds.

***Keywords*:** Antibacterial, Bio compounds, Drug-resistant bacteria, *Staphylococcus aureus*, Phenology, Salvia

Introduction

The increasing emergence of drug-resistant pathogens, especially in healthcare facilities, is a serious problem for people admitted to hospitals, including those with low immunity or chronic diseases (Song et al. 2021). Studies have shown that multi-drug resistance (MDR) bacteria have been developed in human pathogenic microorganisms due to the indiscriminate use of commercial antimicrobial drugs. The wide range of antimicrobial resistance in MDR strains has numerous negative effects, limits effective treatment options, and ultimately increases the economic burden and higher mortality (Yasbolaghi sharahi et al. 2020; Li et al. 2023).

Staphylococcus aureus, one of the most important human pathogens, is mainly responsible for postoperative wound infections, toxic shock syndrome, endocarditis, and food poisoning. It has been reported as the third most common cause of foodborne illness worldwide. Among the bacteria resistant to antibiotics, methicillin-resistant *S. aureus* (MRSA) is one of the main causes of hospital and the community. MRSA infections are very difficult to treat because of their resistance to almost all available clinical antibiotics. For most MRSA strains, glycopeptide drugs such as vancomycin are the only effective antibiotics (Pal et al. 2020). Because of the development of bacterial resistance to commercially available antibiotics, it is necessary to discover alternative, novel, and effective antibacterial compounds from different sources and replace treatment methods based on natural compounds (Aminian et al. 2018; Song et al. 2021). The most important feature of these compounds is their ability to be commercialized without applying chemical changes and their molecular diversity compared to synthetic and semi-synthetic products. The discovery of these substances can be an

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important step in the pharmaceutical, medical, and food industries (Demain, 2006). Therefore, researchers have focused their attention on investigating plants with a wide variety of secondary metabolites that can be a potential source for various antimicrobial, antioxidant, and therapeutic agents (Katiyar et al. 2012; Mgbeahuruike et al. 2017). Studies have shown that the extracts of many plants, especially aromatic species, can inhibit the growth of Gram-positive and Gram-negative bacteria and therefore have a high clinical value in the treatment of resistant bacterial strains (Abedini et al. 2014; Amirian et al. 2018; Aminian et al. 2018). Reactive oxygen species and free radicals, which are natural by-products in metabolic pathways, cause a decrease in the activity of the antioxidant system, change in gene expression, lipid peroxidation, and damage to proteins and DNA in cells and tissues which in turn produce many disorders **(**Aryal et al. 2019; Garcia-Capparos et al. 2021**)**. The concerns about the safety and possible negative effects of synthetic antioxidant compounds and preservatives have led researchers to look for natural alternatives that can be used in various industries.

Medicinal plants are one of the most valuable resources of natural products and contain bio-active and structurally unique compounds that can be used as alternative and suitable sources for the production of natural pharmaceuticals or preservatives (Katiyar et al. 2012; Mgbeahuruike et al. 2017). As rich sources of antioxidants, they have been taken into consideration to protect against the action of free radicals and reduce oxidative damage **(**Aryal et al. 2019; Manuelian et al. 2021). The antioxidant properties of plants are attributed to their bioactive compounds. Flavonoids and phenolic substances can scavenge free radicals and are therefore crucial in nutrition and food sciences **(**Aziz and Karboune 2018**).**

Salvia abrotanoides (Kar.) Sytsma, (formerly *Perovskia abrotanoides*) (Bielecka et al. 2021), is an aromatic plant growing in various regions of Iran. It has been reported that the extract of this medicinal plant exhibited different pharmacological activities including anti-inflammatory (Nassiri-asl et al. 2002), antiseptic, analgesic (Hosseinzadeh and Amel 2001), and cytotoxic (Sairafianpour et al. 2001; Geryani et al. 2016) effects. Previous studies have indicated the antibacterial activity of essential oils from the aerial parts of *S. abrotanoides* on Gram-negative and Gram-positive bacteria such as *Salmonella typhi*, *Staphylococcus aureus*, and *Bacillus cereus* **(**Mahboubi and Kazempour 2009; Ashraf et al. 2014**).** The antimicrobial property of the ethanolic extracts of *P. abrotanoides* aerial parts for vaginal infections has also been demonstrated (Ghafourian and Mazandarani, 2017). The antioxidant activity of aerial parts essential oils of this plant species has been shown by Ashraf et al. (2014).

Considering the importance of discovering and screening native medicinal plants in each country, the present study was conducted to investigate the antibacterial activity of the crude extracts of *S. abrotanoides* in different phenological stages (vegetative, flowering, and seeding) against some drug-resistant pathogens, for the first time. Furthermore, the content of total phenol and flavonoids, as well as the antioxidant potential of extracts obtained from the root and aerial part were evaluated and compared in different developmental stages of the plant. The results of this study can be considered for future practical purposes such as providing formulations to produce new and effective antimicrobial or antioxidant compounds with fewer side effects.

Materials and Methods

Plant material and extract preparation

Aerial parts and roots of *S. abrotanoides* were collected at different phenological stages (vegetative, flowering, and seeding phases) from Tajar in the Northeastern region of Iran. The plants were identified at the Research Center for Plant Sciences, Ferdowsi University of Mashhad, Mashhad, Iran (Herbarium Number: E-1387 FUMH). Samples were air-dried in the shade at room temperature. Extraction was conducted with methanol or ethyl acetate (1:6 W/V) by maceration for 24 hours at room temperature. The extraction process was repeated 3 times and the supernatants were mixed after filtration. The residues were concentrated in a rotary evaporator at 39°C. The crude extracts were dried and stored at -20°C.

Indicator microorganisms

Staphylococcus aureus ATCC 25923, *Bacillus subtilis* PTCC 1156, *Escherichia coli* PTCC 1533, and *Pseudomonas aeruginosa* ATCC 9027, were used as tested bacteria in preliminary screening. The MDR pathogens of methicillin-resistant *Staphylococcus aureus* (MRSA) ATCC 33591, *Pseudomonas aeruginosa* ATCC 2108, *Escherichia coli* ATCC 2452, and *Enterococcus faecium* ATCC 700221 were used in the second screening. Bacterial strains were maintained in glycerol stock at -20°C.

All indicators have been revived on nutrient agar (NA) followed by incubation at 37°C for 24 h.

Characterization of antibacterial activity

The antibacterial activity of crude extracts was tested by the disc diffusion method as described by CLSI guidelines (CLSI, 2012). Bacteria were cultured in Mueller-Hinton agar medium and standardized with a final cell density of approximately 1×10^8 CFU/mL. The extracts were redissolved in methanol or ethyl acetate. Sterile paper discs (6 mm in diameter) impregnated with 30 µL of the crude extracts (at concentrations of 10, 20, and 40 mg/mL) were placed on the inoculated agar and incubated at 37°C for 24 h. Antibacterial activity was evaluated by measuring the diameter of the inhibition zone (mm). All experiments were conducted in triplicate. Amoxicillin (25 µg/disc) and gentamycin (25 µg/disc) were used as positive controls. In the second screening, the plant extracts (30 µL) at a concentration of 40 mg/mL were loaded onto sterile paper discs. Ampicillin (10 µg/disc) and vancomycin (30 µg/disc) were used as positive controls.

Determination of minimum inhibitory concentrations (MIC) and minimum bactericidal concentration (MBC)

MIC values were determined using the broth micro-dilution method. Different concentrations of the extracts (10-100 mg/mL) were prepared by diluting them in Mueller-Hinton broth. At first, 20 µL of the bacterial suspension (10^6 CFU/ ml) was added to 180 µL of each concentration and incubated at 37 °C. Wells containing only medium were used as negative controls while MDR suspension mixed with Mueller-Hinton broth was used as a positive control. After incubation at 37°C for 24 h, 20 µL of 2,3,5-triphenyl tetrazolium chloride (5 mg/mL) was added and incubated at 37°C for 1 hour. The MIC was defined as the lowest concentration of the extracts that prevented the change in medium color. Finally, 20 µL of the suspensions from no color change wells was inoculated on Mueller-Hinton agar plates to determine the MBC (Selim et al. 2022).

Quantification of total phenol content

The total phenol content of the extracts obtained from the roots and aerial parts of *S. abrotanoides* was determined using the Folin-Ciocalteu assay. The extracts were dissolved in methanol (4 mg/mL). Then 2.5 ml of 10% (V/V) Folin-Ciocaltio reagent was added to 1 mL of each extract solution. After 5 minutes, 2 mL of 7.5% (W/V) sodium carbonate (Na_2CO_3) was added. The mixtures were incubated for 60 min at room temperature in darkness, and then the absorbance was measured at 760 nm. Gallic acid was used as a standard, and the content of total phenol in the extract was expressed as milligrams of Gallic acid equivalent per gram of dry weight (mg GAE/g DW) (Aryal et al. 2019).

Quantification of total flavonoid content

The total flavonoids in the extracts were estimated according to the aluminum chloride ($AlCl_3$) colorimetric method. In brief, 500 µL of extracts dissolved in methanol (4 mg/mL) were mixed with 100 µL of 10% (W/V) aluminum chloride, 100 µL of 1 M potassium acetate, and 2800 µL of distilled water. After 30 min, the absorbance of the mixture was measured at 415 nm. Quercetin was employed as a standard, and the flavonoid content was reported as milligrams of Quercetin equivalent per gram dry weight (mg QE/g DW) (Chang et al. 2002).

DPPH Radical Scavenging activity

The ability of extracts to scavenge 2,2-diphenyl-1-picrylhydrazyl (DPPH) free radicals was determined spectrophotometrically. Briefly, 1000 μL of the samples at a concentration of 400 μg/mL was mixed with 3 mL of DPPH methanolic solution (0.004% W/V). After 30 minutes of incubation in the dark at room temperature, the absorbance was measured at 517 nm. Inhibition of DPPH free radical in percentage was calculated as:

Radical Scavenging activity (%) = $[(A_{Control} - A_{Sample}) / A_{Control}) \times 100]$

where $A_{Control}$ and A_{Sample} are the absorbance values of the control and test samples, respectively (Molyneux 2004). Ascorbic acid (100 µg/mL) was used as positive control.

Statistical Analysis

All experiments were conducted with three replications. Statistical analysis was performed using Statistica software (version 12). The data were analyzed using analysis of variance (ANOVA) and the means were compared using Duncan's multiple range test. Differences between means were considered significant at $p \leq 0.05$.

Results

In the initial experiment, a significant difference was observed in the diameter of the inhibition zone

caused by ethyl acetate and methanol extracts of *S. abrotanoide* obtained at different phenological stages against tested bacteria (Tables 1 and 2). Gram-positive bacteria strains were found to be more sensitive than Gram-negative ones to the crude plant extracts. In general, *S. aureus* was the most sensitive and *P.aeruginosa* was the most resistant strain.

The ethyl acetate extract of the root in the seeding stage at a concentration of 40 mg/mL had the most potent effect against *S. aureus* (inhibition zone diameter = 17.6 mm), which was stronger than gentamycin and similar to amoxicillin. The investigated extracts were unable to inhibit the growth of *E. coli* and *P. aeruginosa*, significantly. Moreover, the methanol extracts of the roots obtained at vegetative phase and the aerial part methanol extract at the flowering stage, both at concentration of 40 mg/mL, had comparable effects to gentamicin against *B. subtilis***.** Among aerial parts extracts, the highest inhibitory zone was recorded for the methanolic extract from the vegetative phase against *S. aureus*, which had the same effect as gentamicin. In general, the roots exhibited higher antibacterial activity than the aerial parts. Besides, the results revealed significant variations in antibacterial activity between the extracts of this plant at three developmental stages. The inhibitory effect of the extracts was lower in the vegetative stage compared to the flowering and seeding phases. The effect of the solvent was less compared to the organ type and plant growth stage. The difference between ethyl acetate and methanol was not great, although ethyl acetate showed a slightly better effect than methanol.

Table 1. Inhibition zone diameter (mm) of ethyl acetate and methanol extracts of *S. abrotanoides* roots obtained at different phenological stages against some bacterial strains. (n=3, mean ± SD).

Phenological stage	Extract	Concentration (mg/mL)	Bacteria			
			E. coli	***P.aeruginosa***	***S. aureus***	***B. subtilis***
vegetative	Ethyl acetate	10	-	-	13.00 ± 0.58	9.33 ± 0.67
		20	-	-	12.00 ± 0.40	11.67 ± 0.40
		40	-	-	11.67 ± 1.70	12.33 ± 1.60
	Methanol	10	-	-	11.67 ± 0.60	-
		20	7.67 ± 0.20	-	13.67 ± 0.20	11.00 ± 1.7
		40	9.00 ± 0.70	-	14.33 ± 1.2	12.67 ± 0.67
Flowering	Ethyl acetate	10	-	-	14.00 ± 1.00	-
		20	-	-	14.00 ± 1.4	-
		40	-	-	13.67 ± 0.60	-
	Methanol	10	-	-	14.00 ± 0.58	-
		20	-	-	15.33 ± 0.33	-
		40	-	-	16.00 ± 0.58	-
Seeding	Ethyl acetate	10	-	-	17.33 ± 1.60	-
		20	-	-	16.33 ± 0.70	-
		40	-	-	17.66 ± 0.40	-
	Methanol	10	-	-	9.67 ± 0.20	-
		20	7.67 ± 0.20	-	13.33 ± 0.33	-
		40	8.00 ± 0.00	-	16.00 ± 0.58	-
Amoxicillin			8.33 ± 0.57	16.00 ± 1.7	17.67 ± 0.57	-
Gentamycin			14.33 ± 0.58	10.67 ± 0.58	13.33 ± 0.58	13.33 ± 0.58

A dash (-) indicates no antimicrobial activity.

Table 2. Inhibition zone diameter (mm) of ethyl acetate and methanol extracts of *S. abrotanoides* aerial parts obtained at different phenological stages against some bacterial strains. (n=3, mean ± SD).

			Bacteria			
Phenological stage	**Extract**	**Concentration (mg/mL)**	***E. coli***	***P.aeruginosa***	***S. aureus***	***B. subtilis***
vegetative	Ethyl acetate	10	-	-	10.33 ± 0.40	-
		20	9.00 ± 0.70	-	10.67 ± 0.70	-
		40	9.00 ± 0.58	-	12.67 ± 1.70	-
	Methanol	10	-	-	8.33 ±0.20	-
		20	7.67 ± 0.33	-	12.00 ± 1.4	-
		40	9.00 ± 0.70	-	13.33 ± 0.70	-
Flowering	Ethyl acetate	10	-	-	7.00 ± 0.70	-
		20	-	-	10.33 ± 0.33	-
		40	-	-	11.33 ± 1.60	-
	Methanol	10	-	-	8.33 ± 0.20	10.00 ± 0.58
		20	-	8.00 ± 0.70	8.67 ± 0.20	11.67 ± 0.40
		40	-	8.00 ± 0.57	10.67 ± 0.70	13.00 ± 0.70
Seeding	Ethyl acetate	10	-	-	9.33 ± 0.70	-
		20	9.00 ± 0.58	-	10.67 ± 0.70	-
		40	9.00 ± 0.30	-	12.33 ± 0.90	-
	Methanol	10	-	-	9.00 ± 0.30	-
		20	-	-	10.00 ± 0.60	-
		40	-	-	11.00 ± 0.70	-
Amoxicillin			8.33 ± 0.57	16.00 ± 1.7	17.67 ± 0.57	-
Gentamycin			14.33 ± 0.58	10.67 ± 0.58	13.33 ± 0.58	13.33 ± 0.58

A dash (-) indicates no antimicrobial activity.

Among four drug-resistant bacteria, MRSA showed the most sensitivity to the crude extracts (Table 3). The ethyl acetate extract of the roots in the seeding phase had the largest zone of inhibition (16.33 mm) against this strain, which was similar to vancomycin (Figure 1). The extracts were unable to inhibit the growth of drug-resistant *E. coli* and drug-resistant *P. aeruginosa*. The data indicated that *E. faecium* had the highest inhibitory zone of 10.67 ± 0.7 mm in the treatment of root ethyl acetate extract obtained from the vegetative stage.

There was no significant difference between the flowering and seeding stages in antibacterial properties. The vegetative stage demonstrated lower antibacterial activity against MRSA than the other stages. Similar to the result obtained in the initial screening, the in vitro antibacterial activity of the roots was more than the aerial parts. The MIC and MBC values of the *S. abrotanoides* extracts are given in Table 4. Among the tested bacteria, MRSA was more susceptible to the extracts. The ethyl acetate extract of the roots in the seeding stage displayed the lowest MIC and MBC values for the MRSA strain (30.33 and 40.00 mg/mL, respectively).

Figure 1. Antibacterial activity of *S. abrotanoides* root ethyl acetate extract at seeding (SRE) and flowering stage (FRE) stage against MRSA compared to vancomycin.

Table 3. Inhibition zone diameter (mm) of ethyl acetate and methanol extracts (40 mg/mL) of the roots and shoots of *S. abrotanoides* obtained at different phenological stages against drug-resistant bacteria. (n=3, mean ± SD).

			Bacteria			
Plant organ	**Phenological stage**	**extract**	***E. coli***	***P. aeruginosa***	***MRSA***	***E. faecium***
Root	Vegetative	Ethyl acetate	7.33 ± 0.6	8.33 ± 0.9	12.67 ± 0.3	10.67 ± 0.7
		Methanol	-	7.67 ± 0.7	13.33 ± 0.2	8.33 ± 0.9
	Flowering	Ethyl acetate	-	-	13.67 ± 0.9	-
		Methanol	-	-	12.67 ± 0.4	-
	Seeding	Ethyl acetate	7.00 ± 0.3	-	16.33 ± 1.7	7.67 ± 0.9
		Methanol	8.33 ± 0.2	-	15.67 ± 0.2	9.67 ± 0.4
Shoot	Vegetative	Ethyl acetate	8.33 ± 0.2	-	11.67 ± 0.6	9.33 ± 0.4
		Methanol	-	-	9.00 ± 0.4	-
	Flowering	Ethyl acetate	8.67 ± 0.3	7.33 ± 0.7	9.33 ± 0.3	8.00 ± 0.9
		Methanol	8.00 ± 0.9	8.00 ± 0.7	11.33 ± 0.2	7.67 ± 0.7
	Seeding	Ethyl acetate	-	-	12.67 ± 1.2	9.67 ± 0.4
		Methanol	-	-	8.33 ± 0.9	8.67 ± 0.8
Amoxicillin			8.33 ± 0.7	16.00 ± 0.4	9.00 ± 1.2	10.00 ± 0.2
Vancomycin			18.00 ± 0.4	17.00 ± 0.7	16.00 ± 0.9	15.00 ± 0.3

A dash (-) indicates no antimicrobial activity. MRSA: methicillin-resistant *Staphylococcus aureus.*

Table 4. Minimum inhibitory concentration (MIC, mg/mL) and minimum bactericidal concentration (MBC, mg/mL) of methanol and ethyl acetate extracts of the roots and aerial parts of *S. abrotanoides* against drug-resistant bacteria.

Plant organ	**Phenological stage**	**extract**	***E. coli***		***P. aeruginosa***		***MRSA***		***E. faecium***	
			MIC	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**	**MIC**	**MBC**
Root	Vegetative	Ethyl acetate	65.67	80.00	82.67	82.67	39.67	55.00	37.67	45.00
		Methanol	88.33	93.00	90.33	98.00	37.67	44.00	45.00	46.67
	Flowering	Ethyl acetate	75.33	86.00	90.67	100.00	38.33	45.00	48.67	55.00
		Methanol	72.33	80.00	83.67	87.00	32.33	50.00	56.33	65.00
	Seeding	Ethyl acetate	65.33	70.00	87.67	90.00	30.33	40.00	40.67	50.00
		Methanol	45.33	66.00	82.67	89.00	33.67	41.00	46.67	55.00
Shoot	Vegetative	Ethyl acetate	55.33	66.00	77.33	90.00	41.33	55.00	46.67	61.00
		Methanol	79.67	100.00	85.00	98.00	92.00	97.00	96.00	93.00
	Flowering	Ethyl acetate	98.33	100.00	77.33	88.00	77.33	91.00	72.00	95.00
		Methanol	86.33	95.00	77.33	90.00	47.67	60.00	51.33	55.00
	Seeding	Ethyl acetate	72.00	77.00	77.67	88.00	40.33	50.00	40.67	45.00
		Methanol	68.33	89.00	91.67	100.00	93.33	100.00	90.33	95.00

According to the results, the total phenolic and flavonoid content and also antioxidant potency of the plant differed significantly depending on the organs and phenological stages ($p < 0.05$). The effect of solvent was less (Figure 2). The quantity of total phenol in the methanol and ethyl acetate extracts of *S. abrotanoides* was in the range of 135.14 to 557.51 mg Gallic acid equivalent (GAE)/g DW, whereas total flavonoids content varied from 13.12 to 236.40 mg Quercetin equivalent (QE)/g DW. In all three

physiological development phases of the plant, the aerial parts contained higher total phenol and flavonoid concentrations and also displayed significantly higher DPPH radical scavenging activity as compared to the roots (Figure 1-A, B, C). The ethyl acetate extract of the aerial part in the seeding phase showed the highest total phenol content (557.507 ± 0.317 mg GAE/g DW), flavonoids values (236.40 ± 3.5 mg QE/g DW), and antioxidant activity (92.51 ± 1.25%). The lowest percentage inhibition of DPPH radical (20.43 ± 0.94%) was recorded for ethyl acetate extract of the root in the vegetative phase, while that of the control, ascorbic acid at a concentration of 100 µg/mL, was 88.32 ± 0.784 %.

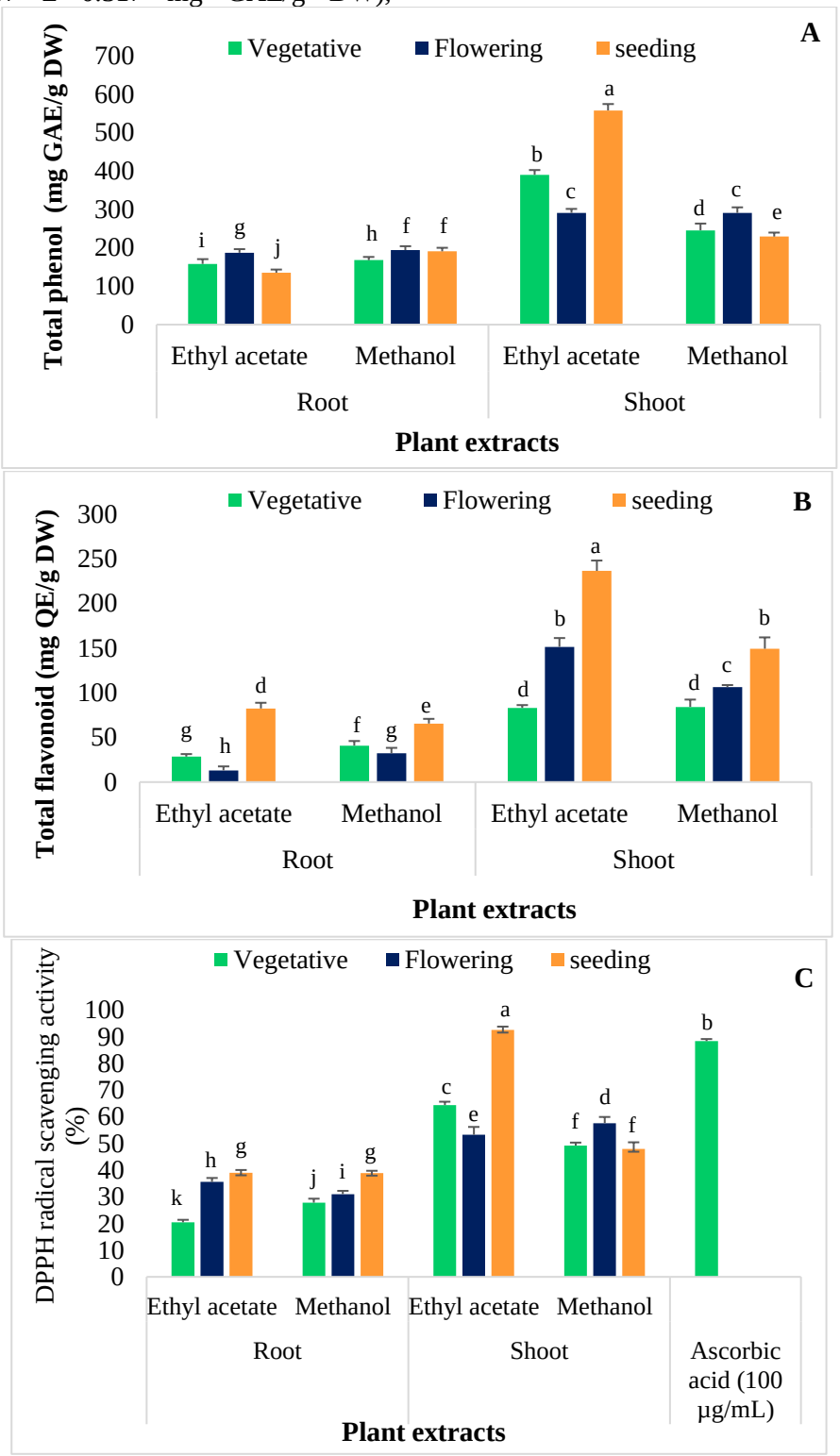

Figure 2. Total phenol (A) and flavonoid (B) content and DPPH radical scavenging activity (C) of methanol and ethyl acetate extracts obtained from the roots and aerial parts of *S. abrotanoides* at different phenological stages. (n=3, mean ± SD). Different letters in each column indicate significant differences ($p \leq 0.05$). GAE: Gallic acid equivalents; QE: Quercetin equivalents.

Discussion

Natural products have long been the most productive source for the development of drugs because of the unmatched availability of chemical diversity (Wang et al., 2015). Several studies have illustrated the therapeutic potential of phytochemical compounds as antibiotics (Nandhini et al., 2022; Li et al., 2023). Currently, considerable attempts have been made to discover plant-derived antibacterial agents against methicillin-resistant *S. aureus* (MRSA). The results of the present study showed the inhibitory effect of crude extracts of *S. abrotanoides* obtained from the roots and aerial parts against *S. aureus* and MRSA. Few natural substances demonstrate potential bactericidal effects against MRSA (Nandhini et al., 2022). There is no report on the antibacterial effect of *S. abrotanoides* against drug-resistant pathogens. Although the antibacterial activity of the aerial part methanolic extract of this plant against *S. aureus* and *P. aeruginosa* has been shown by Abedini et al. (2014) MIC values were reported at 156 and 312 µg/mL, respectively. It has been demonstrated that ethanol extract of the aerial part of *P.abrotanoides* could inhibit the growth of *S. aureus, S. epidermis, Bacillus cereus,* and *Enterococcus faecalis* (Ghafourian and Mazandarani, 2017). Consistent with our results, they also found *S. aureus* more sensitive to the extract than the others, so the diameter of the inhibition zone and MIC value of the extract against this strain was 32.1±0.4 and 45.1 µg/mL, respectively. The antimicrobial activity of *S. abrotanoides* essential oils against *S. aureus* has been reported by Jaderi et al. (2022). Furthermore, the effectiveness of several *Salvia* species such as *S. multicalis, S. chloroleuca,* and *S. brachyantha* extracts on the growth inhibition of *S. aureus* and *B. subtilis* has also been indicated (Bazzaz et al., 2003; Tohma et al. 2016; Asadi- Semnani et al. 2019). It has been reported that alcoholic extracts of *S. officinalis* inhibit the growth of *E. coli*, *S. aureus*, and *P. aeruginosa* (Amirian et al. 2018; Al-Ani et al. 2019). *S. marashica* and *S. caespitosa* methanol extracts inhibit the growth of *S. aureus* and *P. aeroginosa* while it did not show an antimicrobial effect on *E. coli* (Bostanci et al. 2022).

The roots of *S. abrotanoides* are a rich source of tanshinones including cryptotanshinone **(**Sairafianpour et al. 2001). The antibacterial effect of cryptotanshinone has been indicated against *Bacillus subtilis* and *S. aureus* (Lee et al. 1999; Feng et al. 2009). It has been reported that cryptotanshinone could prevent the growth of MRSA and interfere with pyruvate kinase activity, which is the key rate-limiting enzyme in glycolysis (Zhong et al., 2021). Zhao et al. (2021) demonstrated that dihydrotanshinone I inhibits the growth of *S. aureus* and MRSA by damaging the structures of bacterial cell walls and cell membranes, which finally leads to increased permeability of the cell membranes. They also suggested that this metabolite could affect the synthesis of bacterial proteins and result in the loss of the normal physiological function of bacteria. The antibacterial activity of tanshinone I and tanshinone IIA derivatives against Gram-positive bacteria such as *S. aureus* has been shown by Wang et al. (2015). In addition, *S. abrotanoides* is rich in phenolic compounds. So, the antibacterial activity of this species can also be attributed to these substances. The phytochemicals, such as flavonoids, have a good antibacterial effect against MRSA because they could form a complex with the bacterial cell wall, inhibit cell envelope synthesis and ATP synthesis, and damage the membrane structure and bacterial respiratory chain (Nandhini et al., 2022; Jeong et al., 2023). Stafiniak et al. (2021) reported rosmarinic acid as the major phenolic compound in the roots and leaves of *S. abrotanoides* so the content of this metabolite differed during the growth season. The antimicrobial potential of rosmarinic acid is well demonstrated (Ivanov et al. 2022). Although the amount of phenolic compounds was higher in the aerial part of *S. abrotanoides*, the root exhibited higher antibacterial activity. This could be due to the presence of tanshinone in the roots and the synergistic effect of tanshinones and phenolic compounds on bacterial growth. In general, the development of plant-derived antibacterial agents may be a promising strategy against MRSA because of their low side effects, low toxicity, and multi-acting targets (Li et al., 2023). On the other hand, the combined use of herbal extracts can improve the effectiveness of medicinal functions and decrease

side effects by creating synergy and simultaneous effects on several targets (Jeong et al., 2023).

Salvia plants have high antioxidant capacity due to the presence of flavonoids, phenolic acids, and tannins, especially in the aerial part (Al-Ani et al. 2019). According to our results, aerial parts and root extracts of *S. abrotanoides* contained significant amounts of total phenol and flavonoids. The concentration of total phenol in various extracts varied from 135.14 to 557.51 mg GAE/g DW, while flavonoids quantity was in the range of 13.12 to 236.40 mg QE/g DW. Our results were higher than those reported by Ghaderi et al. (2019) who measured the total phenolic content of *P. abrotanoides* aerial parts as 54.9 ± 15.2 mg/g DW. In another study, the flavonoid and phenolic contents of the leaves of *P. abrotanoides* from different populations were reported to be in the range of 2.49 to 4.11 mg QE/g DW and 19.8 to 66.86 mg GAE/g DW, respectively (Ghaffari et al., 2018). This significant difference in reported values could be due to environmental conditions, climatic factors, stage of plant growth and different methods of extraction that significantly affect the phytochemical composition and concentrations (Jordan et al. 2013; Lebedev et al., 2022).

The content of total phenol and flavonoids and well as DPPH free-radical scavenging capacity in the aerial parts of *S. abrotanoides* were much higher than the roots. This could be related to physiology, organ function, and the presence of higher amounts of necessary precursors involved in phenolic biosynthesis in aerial parts due to the photosynthesis process (Belkheir et al. 2016*)*. The results of the present study indicated that total phenol and flavonoid content varied at different plant phenological stages. The extract obtained from the aerial part in the seeding stage had the highest amount of phenolic and flavonoid content and also the highest antioxidant activity. This could be attributed to the age of the plant and also to the increase in temperature and decrease in humidity caused by seasonal changes. It has been shown that the amount and type of phenolic compounds in the plant and their antioxidant properties can be influenced by various factors such as abiotic stresses, location, environmental conditions, season of sample collection, and plant phenological stages (Conner et al. 2002; Jordan et al. 2013).

The extracts of *S. abrotanoides* can be considered as good antioxidant agents, as estimated by the DPPH method. According to our results, as the content of total phenol and flavonoids was higher in the aerial parts of the plant, DPPH free radical scavenging ability was also higher in the extracts. The aerial parts of *S. abrotanoides* contain phenolic compounds, especially rosmarinic acid and salvianolic acids, and the roots contain phenolics and tanshinones (Sairafianpour et al. 2001; Ghaderi et al. 2019; Rostami et al. 2022). The antioxidant effects of these metabolites have been proven (Cao et al. 1996; Park et al. 2009; Khojasteh et al. 2020). In agreement with these results, other *Salvia* extracts such as *S. micristegia, S. brachyantha, S. aethiopis* (Tohma et al. 2016), and *S. officinalis* (Vieira et al. 2020) were shown to have DPPH scavenging activity.

In conclusion, the extract of *S. abrotanoides*, particularly during the seeding stage, could be considered as natural antioxidant and antibacterial agents suitable for future practical purposes such as the development of new drugs or as the main source of food preservative compounds, after further investigations.

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