RESEARCH ARTICLE

Variation in Phenolic Acids, Flavonoids Contents, and Antibacterial Activity of *Prosopis farcta* L. During Phenological Growth Stage

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Abstract

Plants are rich in phenolic chemicals, which have been demonstrated to have antibacterial activities. The current study aims to track changes in phenolic compound accumulation in *Prosopis farcta* organs (leaf, root, flower, pod, and seed) at various growth stages (vegetative, flowering, and seed maturity) and investigate the antibacterial activity of its extracts. At all growth stages, the highest total amount of phenolic compounds and flavonoids was observed in leaves, followed by roots, with the highest quantities measured at maturity. Flowers had the highest concentrations of several phenolics (caffeic acid, coumaric acid, luteolin, and diosmin). In *P. farcta*, there was a strong link between phenolic content and antibacterial action. By using low minimum inhibitory concentrations (MICs), leaf extracts were found to have a robust antibacterial effect against both gram-positive (*Bacillus subtilis* and *Staphylococcus aureus*) and gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*). As a result, *P. farcta* is recommended as a useful source of antibiotic substances for treating bacterial infections.

Keywords: *Prosopis farcta*, antibacterial activity, phenolic compound, flavonoids, minimum inhibitory concentrations.

List of Abbreviations

Abbreviation	Full Term
FW	Fresh weight
DW	Dry weight
GAE	Gallic acid equivalent
RE	Rutin equivalent
MIC	Minimum inhibitory concentration
MBC	Minimum bactericidal concentration
HPLC	High-performance liquid chromatography
SD	Standard deviation

1 Introduction

To date, plants are widely used in folk medicine to treat many disorders in many parts of the world [1, 2]. Among plant bioactive constituents, flavonoids represent a structurally diverse group with well-established roles in human medicine and food applications [3]. Plants as a whole provide a valuable natural source of nutritional and chemical agents [1, 4].

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Among different plant species, the *Prosopis* genus has been extensively used to treat many diseases throughout America, Africa, and Asia countries [5]. *Prosopis farcta* L. is an arid and semi-arid tropical shrub with white, hairy, and sharp spine branches [6].

Previous studies have documented that *P. farcta* has various phenolic compounds with potential applications in treating diabetes [7], as well as anti-inflammatory and antimeasles conditions [2], while antidiabetic activity has also been reported for related species within the *Prosopis* genus [8]. Furthermore, extracts from related *Prosopis* species have been reported to exhibit anticancer, antioxidant, antibacterial, and wound healing effects [9, 10], suggesting similar bioactive potential within the genus. Recently, it has been found that the phenolic metabolites are broadly present in the *Prosopis* species [11].

Phenolics are a large group of secondary metabolites which are not directly used for plant development and reproduction [12]. Phenolic acids are small, simple molecules that comprise a carboxylic acid group on their non-active end [13, 14]. These compounds are a significant class of secondary metabolites [15, 16], whose accumulation in plant cells can be markedly enhanced by elicitors such as methyl jasmonate [17]. They play crucial roles in plant life processes, such as UV-B radiation resistance, pathogen infection defense, nodulation, and pollen fertility [18].

Furthermore, the biosynthesis of phenylpropanoid metabolites is considered as a dynamic and/or periodic process influenced by multiple factors, mainly related to the plants and their habitat [19]. Among these metabolites, flavonoids are particularly responsive to environmental cues such as light and UV radiation, accumulating preferentially in exposed tissues [20]. Biochemical compositions in plants vary from tissue to tissue and strongly depend on the plant phenological growth stages, local geo-climatic and seasonal changes, temperature, humidity, and light [21, 22]. Also, the seasonal fluctuations affect the accumulation of terpenoids and phenolics in *Tithonia diversifolia* leaves and stems [23]. Even though the pharmacological activity of *P. farcta* is well documented, little research has been conducted on the relationship between changes in phenolics as a result of phenological variations and their antibacterial effectiveness.

In addition, several studies have been shown that phenolic acids have a wide range of antibacterial properties and are effective against multi-drug resistant organisms (MDROs) [13]. Plant-derived phenolics, especially phenolic acids, exhibit the potential to kill microorganisms, which can prevent the growth of bacteria as part of the plant antimicrobial system [24]. Other authors have emphasized that inhibitory phenomena on bacteria growth include destabilizing the bacteria cytoplasmic membrane, altering the permeability of the bacteria plasma membrane, inhibiting extracellular microbial enzymes, directly altering microbial metabolism, and depriving microbes of substrates required for growth, which are closely related to phenolic acids [13]. Subsequently, Borges et al. [25] reported that phenolic acids cause changes in bacterial physiochemical surface properties. They also showed the reducing effects of ferulic acid on the hydrophobicity of *Pseudomonas aeruginosa*.

On the other hand, it has been suggested that the level of phenolic compounds and antioxidant activity in *Rumex crispus* L. and *Rumex obtusifolius* L. was principally associated with the type of plant organ excised at different growth stages [26]. Therefore, the current work aimed to evaluate the compositions of phenolics and their antibacterial activity in different tissues at different phenological stages of *P. farcta*.

2 Materials and Methods

2.1 Plant Material

The plant material of *P. farcta* was collected in western Ilam province (33°45'N 46°34'E), Iran. The mean temperature during the sampling period was 14-23 °C. Sampling was conducted using a field-based, stage-stratified collection design, in which plants were harvested at three clearly defined phenological stages: vegetative, floral, and seed maturity. We collected different parts of plants at vegetative stage (leaves, and roots), at the floral stage (leaves, roots, and flowers), and at maturity stage (leaves, roots, pod, and seed). At each stage, biological replicates were collected from multiple individual plants within the same population to minimize plant-to-plant variability. Stage assignment was performed in the field based on standardized morphological criteria (e.g., presence of reproductive structures, pod formation, and seed filling), ensuring consistent discrimination between developmental stages.

All samples were immediately dried at room temperature (25 °C), and then ground into a fine homogeneous powder using a laboratory grinder (ParsKhazar, Model Chilli). To ensure comparability across biochemical and antibacterial assays, all

samples were processed under identical conditions (drying time, grinding procedure, and storage) prior to analysis. The resulting powders were used for biochemical composition analysis and antibacterial activity assays.

2.2 Quantitative determination of total phenolics and flavonoids

Total phenolics contents were assayed based on the Folin-Ciocalteu method [27] with modifications as described by Akkol et al. [28]. In this procedure, 0.2 g samples were extracted by 5 mL of methanol (50%). Then, 5 mL Folin-Ciocalteu reagent, and 4 mL sodium carbonate solution (7%) were added to 1 mL of methanolic extracts. After 2 h, the absorbance was read at 765 nm. Total phenolics were calculated as gallic acid mg g^{-1} DW. Total flavonoids contents were determined using aluminum chloride colorimetric assay [29]. The aforesaid soluble extract (1 mL) was mixed with 4 mL water, then 1.5 mL of 1 M NaOH, 0.5 mL of 5% $NaNO_2$, and 0.5 mL of 10% $AlCl_3$. The absorbance of samples was measured spectrophotometrically at a wavelength of 510 nm. Finally, total flavonoids were analyzed according to rutin equivalents as rutin mg g^{-1} DW.

2.3 Qualitative extraction of phenolic acid and flavonoids using HPLC device

As previously mentioned by Zafari et al. [30] and following established protocols for phenolic extraction and analysis [31], plant samples (0.5 g) were extracted with 3 mL methanol. The methanol extracts were incubated for evaporating solvent. The residues were dissolved in 50 mL acetonitrile and extracted three times with 20 mL hexane for 1 h. The lyophilization extracts were evaporated. After 10 days, the final residues were resolved in 0.5 mL methanol (HPLC grade), and 20 μL of extracts injected into HPLC device (HPLC/DAD (Agilent Technologies 1260 infinity, USA)). The stationary phase was a C18 column (Perfectsil Target ODS-3 (5 mm), 250 $\times$ 4.6 mm; MZ Analysentechnik, Mainz, Germany). The column temperature was maintained at 25 °C. The elution solvents were 2% acetic acid in water (solvent A) and methanol (solvent B) [32], using the following gradient system: 0-2 min 5% B, 2-10 min 25% B, 10-20 min 40% B, 20-30 min 50% B, 30-40 min 100% B, 40-50.0 min 5% B. At a flow rate of 1 mL min^{-1}, phenolic acids were detected using a UV dual-array detector (HP 1040M), which is set at 278, 300, and 340 nm. Flavonoids were assayed using Gudej and Tomczyk's [33] methods. Extraction of flavonoid compounds was performed according to Zafari et al. [30]. To 0.2 g of the fine powder, 1.5 mL of 40% aqueous methanol with 0.5% acetic acid was added. After 3 hours of shaking, samples were centrifuged (12000 rpm, 10 min), and then the supernatant (20 μL) was used for HPLC analysis. The UV detector was set at 254, 280, 300, and 350 nm, and the mobile phase gradient included 0.5 percent phosphoric acid in water (A solvent) and acetonitrile (solvent B). The elution was as follows: 0-30 min 18% B, 30-60 min 67% B, 60-65 min 18% B, 60-70 min 18% B. Chromatography was carried out at a temperature of 25 °C and a flow rate of 0.8 mL min^{-1}. Standard curves of authentic standards were used to quantify the contents of phenolic acids and flavonoids in the extracts of *P. farcta*.

2.4 Anti-bacterial Assay by Disc Diffusion Method

Antibacterial activity was tested using two Gram-positive strains, *Bacillus subtilis* (ATCC465), *Staphylococcus aureus* (ATCC25923), and two Gram-negative strains, *Escherichia coli* (ATCC25922) and *Pseudomonas aeruginosa* (ATCC85327). A concentrated total methanolic extract of phenolics and flavonoids was prepared from the flowers at floral stage, leaves, roots, pods, and seeds at seed maturity stage. The antibacterial activity of these extracts was assayed using the standard disk diffusion method (Kirby-Bauer method) against the mentioned bacteria strains [34]. Stock cultures of these bacteria were grown in a nutrient broth medium at 37 °C for 18 hours (Merck, Germany). Final cell concentrations were adjusted to CFU/mL according to the McFarland turbidity. Then, a lawn culture was accomplished on Mueller-Hinton (MH) agar using sterile cotton swabs (Merck, Germany) plate. 30 μL of various extracts (200 mg mL^{-1} concentration of stock solution) was loaded on sterile 6 mm filter paper discs, and the solvents were allowed to evaporate completely. The plates were incubated at 37°C for 18 hours. Methanol was used as a negative control, and standard antibiotic discs, vancomycin (30 μg) and Streptomycin (30 μg) (PadtanTeb Co.) were used under the same conditions as positive controls. The antibacterial activity was determined by measuring the inhibition zones surrounding discs in mm (with disc paper diameter). This experiment had three independent replications and measurement of anti-bacterial inhibition zones were reported based on the average of the replications.

2.5 Minimal Inhibitor Concentration Assay by Serial Dilution Method

The broth microdilution method was used to calculate minimum inhibitory concentrations (MICs) with slightly modifications [35, 36]. For this test, the extractions were produced at a concentration of 200 mg mL^{-1}. Serial dilutions of methanolic extracts ranging from 0.6 mg mL^{-1} to 20.00 mg mL^{-1} were applied to a 96-well Microtiter plate. Each well containing 0.1 mL double-strength Muller-Hinton Broth (MHB) inoculated with 0.1% of standard bacterial suspension containing 10^7 CFU mL^{-1} of each bacterium and incubated at 37°C and 120 rpm for 18 hours. Positive controls received 30 mg mL^{-1} of penicillin, while negative controls did not have any extract or antibacterial agent. For each dilution of bacteria tested, the microplates were checked for clear or deficiency growth. To assay the bacterial growth, the amount turbidity of the tubes was controlled, and the MIC was determined as the lowest concentration of extract with no visible growth [35–37].

2.6 Minimum Bactericidal Concentration Assay

The drop plate method was used to measure the effects of extracts on minimum bactericidal concentration (MBC) from the microplate wells where no apparent growth occurred. The MBC assay was used to determine the fatal extract concentration to the target bacteria under *in vitro* conditions as a complement to the MIC. A loop of broth culture was spread across the entire surface of the plate from each MIC broth well with no visible growth and incubated at 37°C for 18 hours. The MBC plates were checked for colony growth or lack of change with each dilution subcultured. As a result, MBC was defined as a dilution with no colony growth on agar [35, 36].

2.7 Statistical analysis

To evaluate phenolics in different plant organs at different phenological stages and antibacterial activity for each bacteria strain, the one-way ANOVA followed by Duncan's multiple range test was performed using SPSS (v. 21.0; Statistical Package for Social Science; Chicago, IL, USA). Data are presented as mean ± SD based on three independent biological replicates ($n = 3$), each derived from a separate individual plant. For each biological replicate, measurements were performed in triplicate as technical replicates. Statistical significance was set at $\alpha \leq 0.05$.

3 Results

3.1 Variation of total phenolics and flavonoids in different organs of *P. farcta*

The total phenolics and flavonoids in the leaf, root, flower, pod, and seed at different growth stages are presented in Figure 1. The highest total phenolics and flavonoids were obtained from the leaf extract at all growth stages. The compositions of these metabolites reached the maximum levels at the maturity stage by 89.5 and 54.2 mg GAE g^{-1} DW, respectively (Figure 1).

Also, significant differences of the flavonoids content among the excised parts of *P. farcta* were revealed (Figure 1). At the maturity stage, the flavonoids content for leaves (54.2 mg RE g^{-1} DW; the maximum accumulation of total flavonoids), was 3.48-fold higher than the value determined in the seed (15.59 mg RE g^{-1} DW; the lowest level of the total flavonoids). In the vegetative stage, the flavonoids content in the leaves (38.1 mg RE g^{-1} DW) were 1.78-fold higher than its content in the roots (21.41 mg RE g^{-1} DW). Moreover, a linear regression was applied to show correlation between total phenolics and flavonoids (Figure 2). Results of correlation analysis illustrated highly significant (p-value ≤ 0.05) positive correlation between total flavonoids content with total phenolics accumulation ($R^2 = 0.9483$).

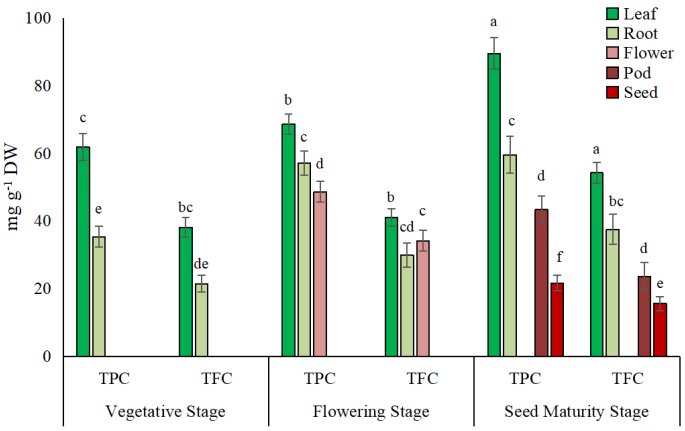

Figure 1. Total phenolic contents (TPCs) and total flavonoid contents (TFCs) in extracts of *P. farcta* samples collected in different phenological stages. Data are means ± SD.

3.2 Chemical profiles of leaves, roots, inflorescences, pods, and seeds by HPLC analysis

The extract of *P. farcta*'s organs was tested for six phenolic acids (gallic acid, caffeic acid, coumaric acid, ferulic acid, cinnamic acid, and salicylic acid) and eight flavonoids (luteolin, vitexin, quercetin, diosmin, myricetin, daidzein, apigenin, and kaempferol). Our results revealed a considerable difference in

Table 1. The content of phenolic acids and flavonoids compounds measured in the extracts of *P. farcta* samples collected in different phenological stages. Data are means ± SD.

Life stage		Vegetative		Floral			Maturity			
		Leaf	Root	Leaf	Root	Flower	Leaf	Root	Pod	Seed
Phenolic acids (μg g^{-1} DW)	Gallic acid	833.4 ± 99c	92.7±25a	1317.5±99b	154.8±38a	523.7±90d	787±80e	478.8±26d	1965±170a	77.8±18a
	Caffeic acid	9.1 ± 1.4a	41.4±6.9c	38.1±1.8cd	50.2±10c	133±25a	108.5±19a	91.1±4.5ab	-	11.5±2e
	Coumaric acid	11.7 ± 1.3b	7.52±0.9c	7.4±1.1c	4.5±0.6c	30±10a	14.8±1.2b	6.1±0.5c	-	-
	Ferulic acid	10.6±1.3c	2.4±0.7d	17.7±3.5b	35±7a	9.7±2c	18.4±4.3b	35±9.1a	10.9±3.2c	7.3±1.9c
	Salicylic acid	1834±216b	56.6±15.6d	2207±186b	77.8±13d	602±51c	4116.1±163a	156.1±30d	35.5±6.5d	80±21d
	Cinnamic acid	16.7±3cd	9.5±1.4de	6.6±0.9e	4.2±0.9e	4.7±1.1e	81.4±13a	17.4±2.2c	51±9b	4.1±1e
Flavonoids (μg g^{-1} DW)	Luteolin	-	1.13±0.19b	-	0.67±0.28bc	1.89±0.15a	-	0.86±0.2b	-	-
	Vitexin	1147±48a	34.6±3cd	913±86b	65.3±9.9c	254±29c	1180±69a	28.3±5.6cd	8.6±2.5d	9.3±1.7d
	Quercetin	53.2±4.5d	126±13bc	114.3±6c	161.8±12b	78.1±15d	173.4±3.3b	213.6±9.6a	152.3±18b	239±20a
	Diosmin	9.7±0.85c	2.8±0.96a	4.8±0.9de	5.5±1.1d	31.9±4.6b	55±7.6a	10.1±2.6c	23.6±4.5b	9.5±1.2c
	Myricetin	8.1±1.5cd	2.7±0.86d	14.7±3.5bc	35±8.1a	16±2.9b	19.2±2.4b	4.9±1.1d	6.4±1.3d	6.3±2.3d
	Daidzein	2.4±1.07b	1.39±0.62bc	4.9±1.05a	2.04±0.75b	4.2±1.04a	1.5±0.3bc	2.8±1.07b	5.2±1.4a	-
	Apigenin	4.2±0.65a	1.15±0.3d	3.2±0.54b	1.95±0.4c	2.5±0.44c	2.27±0.34c	1.23±0.2d	0.75±0.15d	-
	Kaempferol	4.3±0.63b	2.6±0.35c	2.7±0.32c	2.2±0.46d	1.9±0.63cd	7.2±0.75a	1.76±0.4d	-	0.77±0.1e

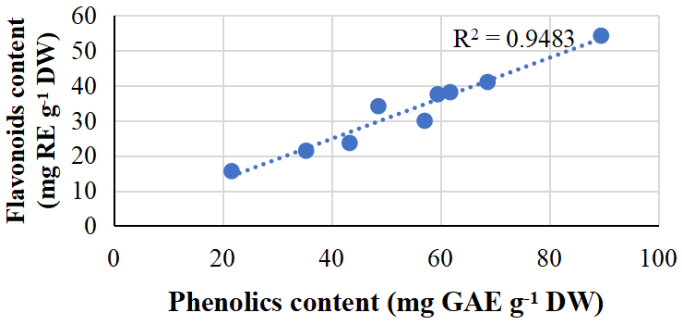

Figure 2. Correlation between total phenolics and flavonoids.

component concentrations between the plant stages (Table 1). Gallic acid was considerably higher in pods by $1965 \pm 170\,\mu g\,g^{-1}$ DW and its content in leaves at the floral stage by $1317.5 \pm 99\,\mu g\,g^{-1}$ DW. The content of caffeic acid in the young developing leaves (vegetative stage) was $9.1 \pm 1.4\,\mu g\,g^{-1}$ DW, whereas in the developed leaves (at the maturity stage) was $108.5 \pm 19\,\mu g\,g^{-1}$ DW. Ferulic acid was detected in both leaves and roots excised during the floral and maturity stages of *P. farcta*. During the maturity stage, cinnamic acid was higher in leaves and pods. The salicylic acid content increased to $4116.1 \pm 163\,\mu g\,g^{-1}$ DW in mature leaves.

Furthermore, leaves collected at all three stages of *P. farcta* growth contained a significant vitexin content. Quercetin levels increased significantly in all organs of *P. farcta*, with the maximum concentration in the seed. ($239 \pm 20\,\mu g\,g^{-1}$ DW). Roots had a high level of myricetin during floral stage, followed by leaves at maturity. Luteolin contents were negligible in flowers and roots, but they were below the detection limit in leaves, pods, and seeds. Flowers have the highest caffeic acid, coumaric acid, luteolin, and diosmin levels. A group of flavonoids, including apigenin, kaempferol, and daidzein, were detected but did not differ significantly during the *Prosopis* growth phases.

3.3 Antibacterial activity of different extracts

For determining antibacterial activity, we used the methanolic extracts of leaf, root, seed, and pod at maturity stage, and also, the methanolic extract of flower at floral stage. As shown in Table 2, the antibacterial activity substantially varied depending on the plant part and bacterial strains. The majority of *P. farcta* extracts were potent against at least three of the microbial strains used in the study. Standard antibiotics such as streptomycin and vancomycin were used as a positive control. The pure methanol was added as negative control. Among different parts, the leaf of *P. farcta* showed the strongest activity against all bacteria tested (e.g., *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa*), with inhibition zone diameters ranging from 12 to 16 mm.

Table 2. Measurement of anti-bacterial inhibition zones of leaf and root extracts of *P. farcta* at maturity stage.

Methanolic Extract of Root concentration (µg mL^{-1})	**Bacteria inhibition zone (mm)**			
	B. subtilis	*S. aureus*	*E. coli*	*P. aeruginosa*
Leaf	16	15	12	12
Root	14	12	12	11
Flower	9	10	8	8
Pod	9	9	8	7
Seed	8	7	6	7
Streptomycin (30 μg)	12	12	18	16
Vancomycin (30 μg)	20	18	8	7
Methanol	3	4	3	2

The leaf extracts excised at maturity stage exhibited higher values than the root extracts due to their higher phenolic content and antibacterial activity. Furthermore, the total methanolic extract from the leaf exhibited stronger antibacterial activity against *B. subtilis* and *S. aureus*, with inhibition zones of 16 and 15 mm, respectively, compared to Streptomycin, which

Table 3. Minimum inhibitory concentration (MIC) and minimum bactericidal activity (MBC) assay of methanolic extract of the *P. farcta* Leaf.

Methanolic Extract of Leaf concentration (μg mL^{-1})	B. subtilis		S. aureus		*E. coli*		P. aeruginosa	
	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
500	-	-	-	-	-	-	-	-
250	-	-	-	-	-	-	-	-
125	-	-	+	-	-	-	-	-
62.5	+	-	+	-	-	-	-	-
31.25	+	+	+	+	-	-	+	-
15.62	+	+	+	+	+	+	+	+
7.81	+	+	+	+	+	+	+	+

(-) represent the inhibition of the growth of bacteria strains.

(+) represent the visible growth of bacteria strains.

Table 4. Minimum inhibitory concentration (MIC) and minimum bactericidal activity (MBC) assay of Methanolic extract of the *P. farcta* root.

Methanolic Extract of Leaf concentration (μg mL^{-1})	B. subtilis		S. aureus		*E. coli*		P. aeruginosa	
	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC
500	-	-	-	-	-	-	-	-
250	-	-	-	-	-	-	-	-
125	-	-	-	-	-	+	-	-
62.5	-	-	-	-	-	+	-	-
31.25	+	+	-	-	+	+	+	+
15.62	+	+	-	+	+	+	+	+
7.81	+	+	+	+	+	+	+	+

(-) represent the inhibition of the growth of bacteria strains.

(+) represent the visible growth of bacteria strains.

had inhibition zones of 12 and 12 mm.

In comparison to other strains, *B. subtilis* is the most susceptible to the extracts. Overall, leaf extracts could be used as a natural antibiotic and alternative treatment for illnesses. We used the total methanolic extracts of leaf and root for the antimicrobial test since it exhibited the largest inhibition zone against all bacteria strains. Tables 3 and 4 show the findings of the antimicrobial assay of *P. farcta* leaf and root extracts, which show that the methanolic extracts of both organs had an inhibitory effect against the four bacteria tested.

The minimum inhibitory concentration (MIC) of leaf extract for the four bacteria *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* were 31.25, 31.25, 62.50, and 62.50 μg mL^{-1}, respectively, while minimum bactericidal activities (MBC) for leaf were 62.50, 31.25, 250, and 125 μg mL^{-1}, respectively (Table 3). In contrast, the minimum inhibitory concentration (MIC) for the root extracts for *B. subtilis*, *S. aureus*, *E. coli*, and *P. aeruginosa* was 62.50, 15.62, 62.50, and 62.50 μg mL^{-1}, respectively. Minimum bactericidal activities (MBC) for roots were 62.50, 31.25, 250, and 62.50 μg mL^{-1} (Table 4). The MIC range for standard vancomycin and Streptomycin was 0.4-4 μg mL^{-1} for all strains.

Aligiannis et al. [38] introduced a classification of plant extracts based on their MIC values: strong inhibition: MIC $<$ 500 μg mL^{-1}; moderate inhibition: 600 μg mL^{-1} $<$ MIC $<$ 1500 μg mL^{-1} and low inhibition: MIC $>$ 1600 μg mL^{-1}. According to this classification, the leaf extract had a substantial inhibitory effect on all bacteria tested. The comparison of MICs and MBCs values provides a more accurate assessment of bioactive substances' antibacterial activities. According to Biyiti et al. [39] findings, a substance is bactericidal if the MBC/MIC ratio is less than 2 and bacteriostatic if the

MBC/MIC ratio is more than 2.

In this study, the level of MIC and MBC is close or equal. Based on the results, we can deduce that the leaf extract had an antibacterial effect on *B. subtilis*, *S. aureus*, and *P. aeruginosa* and a bacteriostatic impact on *E. coli*.

The antibacterial activity of root extracts was lower than that of leaf extracts against bacteria due to the different profiles of phenolic components in these organs.

3.4 Correlation between biological activity and phenolic contents

The effect of total phenolics on antibacterial activity was analyzed based on inhibition zone (mm) (Figure 3). As shown in Figure 3(a), antibacterial activities against *B. subtilis* and *S. aureus* demonstrated a correlation with total phenolics content, with R^2 of 0.7068 and 0.6351, respectively. These results suggest phenolics as active constituents of antibacterial activity against *B. subtilis* and *S. aureus*. According to Figure 3(b), there was a weak correlation of total phenolics content with the antibacterial of *E. coli* ($R^2 = 0.4746$). Also, our data showed that the accumulation of total phenolics had a positive correlation with *P. aeruginosa*, with R^2 of 0.7637.

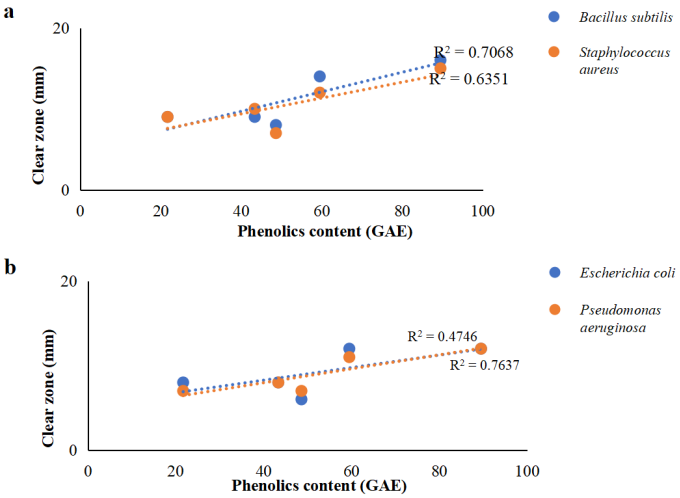

Figure 3. Correlation between antibacterial activity and phenolic contents of P. farcta in response to four strains of bacteria.

4 Discussion

Recently, there has been overwhelming interest in understanding the antibacterial properties of plant extracts, which have several phenolic compounds such as phenolic acids and flavonoids [40, 41]. Also, studies on plant beneficial substances are insufficient due to various plant species and diverse secondary metabolites [16, 40]. A few microbiological reports declare the antibacterial potential of phenolic acids and flavonoids [42, 43].

In this study, we firstly analyzed the effect of phenological stages on the alterations of *P. farcta* metabolites. It was found that the phenological stages can directly affect the amount of total phenolics and flavonoids in *P. farcta* extracts. The highest content of total phenolics and flavonoids was monitored at the maturity stage based on the obtained results. Consequently, total phenolics contents significantly correlated with flavonoids content.

Accordingly, the phenolic acids and flavonoids profiles in *P. farcta* were detected by the HPLC system. As shown in Table 1, HPLC analysis also suggested the presence of six phenolic acids and eight flavonoids in *P. farcta* extracts. The chemical identification of the substances was confirmed using commercial standards, and it was in line with earlier findings [5, 44]. Changes in the contents of phenolic acids and flavonoids indicate that different phenological states are associated with tissue-specific accumulation patterns. Our findings support Chepel et al. [45] studies, which found that the compositions of phenolics and flavonoids depended on the plant development stage, growth, and development stage. In addition to plant species and phenological stages, changes in phenolic profiles are influenced by environmental circumstances [21, 22, 46]. For example, flavonoids involve UV radiation absorption and mostly accumulate in UV-exposed tissues such as leaves and flowers, processing UV protection [47].

Phenolics content rises with leaf age and is lower in the early stages of leaf development, gradually increasing with leaf maturity [48]. Moreover, it has been discussed that phenolic acids show antibacterial activity and function as food preservatives [49].

We tried to explain the antibacterial potential of *P. farcta* extracts. At low concentrations (7.8 and 15.6 μL), no inhibition was determined in the extracts of *P. farcta*. Apparent growth inhibition was observed when 125 μL of the extracts of leaves and roots were used in the culture medium. *B. subtilis* and *S. aureus* revealed sensitivity to the low leaf and root extracts concentration according to inhibition zone (16 and 15 mm, respectively). These results agreed with Tian et al. [50], who reported that Gram-positive pathogens, such as *S. aureus* had a sensitivity to the low dose the

extracts of berry plants leaves. Also, the involvement of phenolic compounds in antibacterial activities against selected Gram-positive and Gram-negative strains was documented by the previous investigation in several plant species [51, 52].

The number of hydroxyl groups in phenolic compounds can drastically affect their antibacterial properties, illustrating the potent inhibition of Gram-positive strains caused by coumaric acid and caffeic acid in this research. Consistent with our data, Tian et al. [50] and Puupponen-Piimiä et al. [53] suggested that hydroxyl groups in phenolic compounds are directly associated with antibacterial activities against Gram-positive strains.

Bourab-Chibane et al. [54] explained that phenolics treatment could change bacterial polarity by affecting the rate of bacteria surface electron acceptors on both gram-positive (increased acceptor components) and gram-negative (decreased acceptor components) strains. Also, they observed that an enhancement in phenolic acid concentration could dramatically increase the percentage of cell membrane damage by releasing intracellular K^+. These effects were much more significant in Gram-negative strains than in Gram-positive strains. Our results show that an increase in phenolic acid compounds did not lead to inhibition of the growth of Gram-negative bacteria. As a result, these findings agreed with Bourab-Chibane et al. [54]. The antibacterial mechanism of different phenolic compounds is not fully understood [50]. Recent speculation is confirmed that the inhibitory effects of phenolic compounds are related to the outer layers of Gram-positive and Gram-negative bacteria [50]. Antibacterial elements can quickly devastate Gram-positive bacteria's cell walls, leading to cytoplasm leakage and its coagulation [41]. The antibacterial activity of phenolic acids is determined by pH, pKa, lipophilicity, hydroxyl and methoxy group substitutions, and side-chain saturation. Antimicrobial activity is higher in oxidized phenols with more hydroxyls than in less oxidized phenols [55].

On the other hand, the biological activity of flavonoids can be related to the catechol group in the B-ring of their constituting skeleton by comparing the inhibition efficacy of myricetin, quercetin, kaempferol, luteolin, and apigenin against the mentioned bacteria strains. Quercetin probably has a potent antibacterial property among other flavonoids in the extracts of leaves and roots. This suggestion was in line with the findings of Kumar and Pandey [56], who reported that the antioxidant effects of flavonoids vary depending on the catechol group in their structure.

Apigenin disrupts energy and nutrition exchange by altering the permeability of bacteria's outer and cytoplasmic membranes, inhibiting peptidoglycan synthesis. Apigenin is effective against gram-positive bacteria such as *S. aureus* and *B. subtilis* [57]. Also, they reported that quercetin could inhibit the formation of NO and slows the rate of programmed cell death, protecting the host against bacterial infections. Consistent with the high gallic acid content detected in *P. farcta* extracts, particularly in pods and floral-stage leaves (Table 1), gallic acid has demonstrated potent inhibitory effects against various bacterial pathogens; for instance, Díaz-Gómez et al. [58] reported strong inhibition of *H. pylori* strains by gallic acid and catechin, further supporting the broad-spectrum antibacterial relevance of this phenolic compound.

Our observations displayed significant antibacterial properties of the total methanolic extracts (phenolic acids + flavonoids) against clinical strains of Gram-positive (*B. subtilis* and *S. aureus*) and Gram-negative pathogens (*E. coli* and *P. aeruginosa*). According to the MIC classification proposed by Aligiannis et al. [38], all tested extracts demonstrated strong inhibitory activity, with MIC values below 500 μg mL^{-1}. Consistently, the minimum inhibition zone was observed against *P. aeruginosa*, which is well known for its intrinsic resistance to a broad range of antimicrobial agents.

In brief, our outcomes emphasize that different phenology stages can significantly change the quality of *P. farcta* organs' extracts and their antibacterial activity. Regarding the antibacterial properties of this plant, the extraction of mature leaves and roots of *P. farcta* can serve as a good source of Phyto-antibacterial and phenolic compounds with good antioxidant capacity.

5 Conclusion

The present work shows the importance of phenological stage as one of the major factors influencing phytochemical constituents and antibacterial activity of *P. farcta*. These findings validate the assumption that changes in development have an effect on secondary metabolites, such as phenolic acids and flavonoids, whose levels increase significantly at the mature stage. The impact of phenological stage was significantly reflected in both quantitative (total phenolics and flavonoids) and

qualitative (HPLC-identified compounds) shifts in extract composition. From a functional perspective, the highest antibacterial activity was achieved by the extracts of mature stage of samples, particularly against Gram-positive strains such as *B. subtilis* and *S. aureus*. The limited effect observed against Gram-negative strains further suggests that outer membrane barriers restrict phenolic penetration, highlighting a structure–activity relationship between compound chemistry and microbial susceptibility. Collectively, these findings propose that phenological regulation of phenolic biosynthesis is directly linked to antibacterial potency in *P. farcta*. Therefore, harvesting at the mature stage can be considered optimal for maximizing bioactive yield and antimicrobial efficiency. This work provides a mechanistic basis for exploiting developmental stage as a practical strategy to enhance the value of medicinal plants as natural sources of phenolic-based antibacterial agents.

Data Availability Statement

Data will be made available on request.

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Conflicts of Interest

Mostafa Sagharyan served as an Editorial Board Member of the *Plant Innovation Journal* at the time of manuscript submission. To ensure the integrity of the peer-review process, Mostafa Sagharyan was not involved in the editorial handling, peer review, or decision-making process for this manuscript, which was handled independently by another editor. The remaining author declares no conflicts of interest.

AI Use Statement

The authors declare that no generative AI was used in the preparation of this manuscript.

Ethical Approval and Consent to Participate

Not applicable.

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