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Comparative In Vitro Evaluation of Antibacterial Activity of Ethanolic Extracts of *Dorema aucheri*, *Allium rotundum*, and *Flacaria vulgaris* Collected from Kermanshah Province, Iran

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ABSTRACT

Background*:* Antibiotic resistance among pathogenic bacteria has become a global health concern, prompting the search for alternative antimicrobial agents. Native medicinal plants such as *Dorema aucheri*, *Allium rotundum*, and *Falcaria vulgaris*, long used in traditional Iranian medicine, are underexplored despite their potential bioactivity. This study aimed to evaluate and compare the antibacterial effects of ethanolic extracts from these plants, collected from Kermanshah Province (Dallahoo mountains), Iran, against five clinically relevant bacterial strains.

Methods: Ethanolic extracts of tree native medicinal plants were prepared via percolation and concentrated using rotary evaporation. Antibacterial activity against five standard strains (*S. aureus*, *E. faecalis*, *E. coli*, *P. aeruginosa*, and *K. pneumoniae*) was assessed using two complementary in vitro techniques: the well diffusion assay to evaluate inhibition zones, and broth microdilution to determine minimum inhibitory concentrations (MICs). Statistical analyses including Kruskal-Wallis and ANOVA were applied to identify significant differences among plant extracts and bacterial responses.

Results: *D. aucheri* exhibited the most potent MIC values against Gram-positive bacteria, particularly *E. faecalis* (0.46 mg/mL), while *A. rotundum* demonstrated broad-spectrum activity in the well diffusion assay, effectively inhibiting all five bacteria, including *K. pneumoniae* and *P. aeruginosa*. *F. vulgaris* showed selective activity, most notably against *S. aureus* (20 mm zone; MIC = 2.87 mg/mL). Notably, *E. faecalis* was the most susceptible strain overall, with significantly larger inhibition zones across extracts compared to *K. pneumoniae* and *P. aeruginosa* ($p < 0.001$).

Conclusion: This study offers novel comparative data on the antibacterial properties of three ethnobotanically significant Iranian plants. The promising activity, especially of extracts from *D. aucheri* and *A. rotundum*, underscores the potential of Iran's phytodiversity in addressing antibiotic resistance. Future studies should further explore regional phytochemical variability and isolate active constituents for therapeutic development.

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Introduction

Bacterial infections and epidemics continue to pose a major threat to global public health, exacerbated by the rise of multidrug-resistant (MDR) strains due to the extensive use and misuse of antibiotics in human and veterinary medicine (1, 2). According to the World Health Organization (WHO), antimicrobial resistance is one of the top ten global public health threats facing humanity (3, 4). As conventional antibiotics gradually lose efficacy, there is a growing need to explore alternative antibacterial agents with improved safety profiles and reduced resistance potential (5, 6).

In recent years, plant-derived bioactive compounds have gained increasing attention as promising candidates for novel antimicrobial therapies (7). Medicinal plants, through their secondary metabolites, have evolved to combat microbial pathogens and ecological stressors. These metabolites—such as flavonoids, alkaloids, saponins, tannins, terpenoids, and phenolic acids—exhibit broad-spectrum antibacterial, antioxidant, and anti-inflammatory activities (8, 9). As reported by the WHO, approximately 80% of the global population relies on traditional plant-based remedies for primary healthcare, emphasizing the importance of scientifically validating traditional knowledge to support the development of new therapeutics (10, 11).

Among the diverse flora of Iran, the western region—particularly Kermanshah Province—hosts a variety of aromatic and medicinal plants long used in traditional medicine. Located in the Zagros mountain range, this region provides a unique ecological niche that supports the growth of species with potential bioactivity (12, 13). In this context, three ethnobotanically significant species were selected for evaluation: *Falcaria vulgaris* (locally known as Paghazeh), *Dorema aucheri* (Zoo), and *Allium rotundum*.

F. vulgaris, a member of the Apiaceae family, is a fast-growing herb commonly used as a spring vegetable in western Iran (14). Traditionally, it has been used for treating gastrointestinal ailments, stomach ulcers, kidney and bladder infections, and superficial wounds. Phytochemical analyses have reported the presence of saponins, tannins, and essential oils such as spathulenol and carvacrol—compounds with known antiseptic activity (14, 15). *D. aucheri*, also belonging to the *Apiaceae* family, is a well-known medicinal plant whose extract contains high levels of flavonoids, conferring both antimicrobial and antioxidant properties. Traditionally, it has been used to reduce blood triglycerides and cholesterol, relieve pain, and enhance immune cell function. Previous studies have shown that essential oils from *D. aucheri* can interfere with quorum sensing and virulence factors in pathogens such as *Pseudomonas aeruginosa* (16, 17). *A. rotundum*, from the *Alliaceae* family, is related to wild garlic and has been used in Iranian traditional medicine for managing gastrointestinal and respiratory infections. Its organosulfur compounds are thought to play a central role in its antimicrobial effects (18, 19).

Despite their widespread use in traditional Iranian medicine, scientific evaluation of the antibacterial properties of these native medicinal plants—particularly those endemic to the western province of Kermanshah, where they have long been used in folk remedies—remains limited. While a few studies have investigated the crude extracts of these species, there is a notable lack of comprehensive comparative analyses that simultaneously assess their antibacterial efficacy. Moreover, no previous study to date has examined the inhibitory effects of *F. vulgaris*, *D. aucheri*, and *A. rotundum* extracts against five clinically relevant bacterial strains with distinct biological characteristics (Gram-positive and Gram-negative). This critical gap underscores the need for rigorous experimental validation. The present study was therefore designed to address this gap by evaluating and comparing the in vitro antibacterial activity of ethanolic extracts of these three traditionally used plants, all indigenous to the Kermanshah region in western Iran. In doing so, the study not only explores their antibacterial potential but also provides new scientific evidence

to support their ethnopharmacological applications and identifies promising candidates for the development of plant-based antimicrobial agents.

Materials and Methods

Bacterial Strains

Five standard bacterial strains were employed in this study for comparative analysis and validation purposes. These included Escherichia coli ATCC 25922, *Staphylococcus aureus* ATCC 25923, *P. aeruginosa* ATCC 27853, *Klebsiella pneumoniae* ATCC 10031, and *Enterococcus faecalis* ATCC 9854. Each strain was obtained from recognized international collections, ensuring standardization and reliability of the experimental procedures. The strains were stored and cultured according to the manufacturer's recommended protocols and maintained under appropriate laboratory conditions to preserve their viability and genetic integrity throughout the study.

Plant Collection Area

The plants used in this study—*Falcaria vulgaris*, *Allium rotundum*, and *Ferula aucheri*—were obtained from Kermanshah Province in western Iran. This Province covers an area of approximately 2.5 million hectares, constituting about 1.5% of Iran's total land area, and is situated between 33°40´ and 35°10´ N latitudes and 45°30´ and 48°20´ E longitudes (Figure 1). The region features diverse topography, including plains, hills, and mountainous terrains, with land use spanning forests, rangelands, and agricultural lands. The mean annual precipitation and temperature are 480 mm and 17.7 °C, respectively, with altitudes ranging from 270 m to 3,350 m above sea level. Kermanshah Province lies between the Iranian plateau and the Mesopotamian plain, predominantly covered by the Zagros mountain chains, and shares climatic characteristics with the Mediterranean region (20). The plants were collected from their natural habitats on the Dallahoo mountains and the highlands of Kermanshah. To ensure the preservation of their bioactive compounds, the specimens were dried under open-air conditions in a shaded environment following collection.

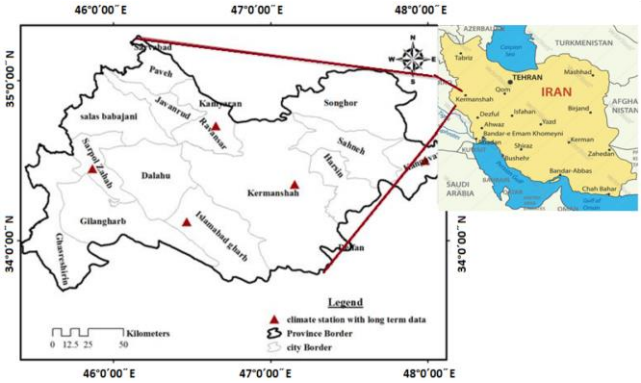

Figure 1. The map of the study area displays the locations of climate stations within Kermanshah Province (20).

Preparation of Ethanolic Extract and Initial Concentration

The total plant extract was prepared using the percolation method (21). The percolation process was carried out using a modified TIMATIC Duo percolator (Tecnolab srl, Spello, Italy). Fifty grams of each plant's dried powder was soaked in 50 mL of ethanol for 2 hours, with intermittent shaking to ensure proper extraction. Following this, the sample was further extracted using 70% ethanol through the percolation technique. The resulting extract was collected, evaporated to dryness using a rotary evaporator, and subsequently filtered and sterilized using a 0.2 µm filter (Membrane: PES - VWR®). The sterilized extracts were stored in dark conditions at -20 °C and used within one week to preserve their integrity.

To prepare the initial concentration, 5 mL of each pure plant extract was added to dimethyl sulfoxide (DMSO) and mixed thoroughly until completely dissolved, resulting in a homogeneous solution. For *Allium rotundum* and *Falcaria vulgaris*, 15 mL of DMSO (3%) was used to dissolve the extract, while for *Dorema aucheri*, 25 mL of DMSO (3%) was required. Consequently, the primary stock

concentrations were 250 mg/mL for *Allium rotundum* and *Falcaria vulgaris*, and 166.5 mg/mL for *Dorema aucheri*.

Serial twofold dilutions of each sample in DMSO were prepared to achieve concentration ranges of 0.48–250 mg/mL for *Allium rotundum* and *Falcaria vulgaris*, and 0.37–166.5 mg/mL for *Dorema aucheri*. These solutions were used for subsequent experimental assays.

Antimicrobial Activity

The evaluation of the antibacterial properties of the plant extracts was carried out following procedures described in previously published studies, with slight modifications (22-25). Two complementary techniques were employed: the well diffusion method to determine the zones of inhibition (25), and the agar dilution method to establish the Minimum Inhibitory Concentration (MIC) (24, 26). All tests were conducted in triplicate, and mean values were reported for final analysis.

Well Diffusion Assay

Muller-Hinton Agar (MHA; Merck, Germany) plates were prepared following the manufacturer's instructions and allowed to solidify at room temperature for 30 minutes. The test bacterial strains were adjusted to a standardized inoculum density of 1.5×10^8 CFU/mL, equivalent to 0.5 McFarland standard, and were evenly spread across the agar surface using sterile cotton swabs. Wells of 6 mm diameter were aseptically bored into the agar using a sterile cork borer. Specific volumes of the plant extracts were introduced into the wells at concentrations ranging from 0.48 to 250 mg/mL for *Allium rotundum* and *Falcaria vulgaris*, and from 0.37 to 166.5 mg/mL for *Dorema aucheri*, based on the prepared stock solutions

The plates were allowed to stand at room temperature for 1 hour to facilitate diffusion, followed by incubation at 37 °C for 24 hours. Zones of inhibition surrounding the wells were measured using a metric ruler and recorded in millimeters. A zone of inhibition ≥12 mm was considered indicative of strong antibacterial activity, in line with previous studies. Gentamicin (10 µg/disc) and 3% DMSO served as the positive and negative controls, respectively.

Agar Dilution Method

To determine the MIC, 75 mL of MHA was distributed into ten separate Erlenmeyer flasks, sterilized by autoclaving, and maintained at 50 °C. Subsequently, 5 mL of each plant extract at mentioned concentrations was added to the corresponding flask, gently mixed, and poured into sterile Petri dishes. The media were allowed to solidify for 30 minutes at room temperature. Overnight bacterial cultures were prepared in sterile Muller-Hinton Broth and diluted to a final turbidity of 1.5×10^5 CFU/mL. A 20 µL aliquot of the standardized inoculum was carefully spotted onto the surface of each agar plate. The plates were incubated at 37 °C for 24 hours. The MIC was defined as the lowest concentration of the plant extract that completely inhibited visible bacterial growth.

Statistical Analysis

All statistical analyses were performed using SPSS version 26.0 (IBM Corp., Armonk, NY). Due to the small sample size and the non-normal distribution of the data (confirmed by the Shapiro–Wilk test), non-parametric methods were applied. The Kruskal–Wallis H test was used to evaluate differences in antibacterial activity (inhibition zones and MIC values) among the three plant extracts across bacterial strains. Where significant differences were detected, Dunn's post-hoc test with Bonferroni correction was applied for pairwise comparisons. A significance threshold of $p < 0.05$ was used for all tests. All numerical values presented in the results represent the mean of three independent experimental replicates.

Results

The ethanolic extracts of *A. rotundum*, *F. vulgaris*, and *D. aucheri* exhibited varying degrees of antibacterial activity against the five standard bacterial strains, as determined by the well diffusion method. A clear concentration-dependent inhibition pattern was observed in all cases, with higher extract concentrations generally producing larger zones of inhibition (Table 1). Statistical analysis revealed significant differences in the antibacterial efficacy among the three plant extracts (Kruskal-Wallis $H = 18.74$, $p < 0.001$). Post-hoc pairwise comparisons indicated that *D. aucheri* produced significantly greater inhibition zones than *F. vulgaris* ($p = 0.004$) and *A. rotundum* ($p = 0.032$) when averaged across all bacterial strains. However, *A. rotundum* demonstrated broader-spectrum activity, significantly outperforming *F. vulgaris* against *K. pneumoniae* and *P. aeruginosa* ($p = 0.021$ and $p = 0.047$, respectively).

Among the three extracts, *D. aucheri* demonstrated the most potent antibacterial activity overall, particularly against *E. faecalis*, for which the inhibition zone reached an exceptional 55 mm at 166.5 mg/mL. This value markedly exceeded all other inhibition zones recorded across the study. In contrast, *P. aeruginosa* and *K. pneumoniae* showed complete resistance to *D. aucheri*, exhibiting no measurable inhibitory response at any concentration tested (zone = 0 mm across all concentrations) (Table 2).

A. rotundum displayed broad-spectrum antibacterial activity, effectively inhibiting all five bacterial strains, including *Klebsiella pneumoniae*, which was resistant to *F. vulgaris* and *D. aucheri*. The ethanolic extract of *A. rotundum* demonstrated inhibitory activity against all five tested bacterial strains at a concentration of 250 mg/mL, with its effect on *Pseudomonas aeruginosa* being comparable to that of Gentamycin. This extract produced inhibition zones ≥25 mm against *K. pneumoniae* at the highest concentration. The lowest effective concentration with ≥12 mm inhibition for most strains was approximately 15.6 mg/mL (Table 3).

F. vulgaris exhibited moderate antibacterial activity. Although effective against *S. aureus*, *E. faecalis*, *P. aeruginosa*, and *E. coli*, its extract failed to inhibit *K. pneumoniae* at any concentration. Inhibition zones for sensitive strains were generally smaller compared to *A. rotundum* and *D. aucheri*, indicating a weaker overall antibacterial profile (Table 4).

In terms of bacterial susceptibility, *E. faecalis* was the most sensitive strain across all extracts, showing the highest inhibition zone (55 mm) in response to *D. aucheri* at 166.5 mg/mL. One-way ANOVA confirmed statistically significant differences in inhibition zones across bacterial strains ($F(4, 55) = 9.63$, $p < 0.001$). Tukey's post-hoc test showed that *E. faecalis* had significantly larger inhibition zones compared to *K. pneumoniae* ($p < 0.001$) and *P. aeruginosa* ($p = 0.006$). *S. aureus* also demonstrated consistent and statistically significant susceptibility to all three extracts ($p = 0.015$ when compared to *P. aeruginosa*).

When compared to the positive control (Gentamycin), the ethanolic extracts exhibited varying degrees of proximity in their antibacterial effects (Table 5). Notably, *F. vulgaris* demonstrated an inhibition zone of 19 mm against *P. aeruginosa*, which was remarkably close to that of gentamycin (18 mm), indicating comparable efficacy. Similarly, *A. rotundum* showed a 25 mm inhibition zone against *K. pneumoniae*, only 3 mm less than gentamycin (28 mm).

In contrast, for *S. aureus*, *E. faecalis*, and *E. coli*, the inhibition zones produced by the extracts were 6-7 mm smaller than those observed with gentamycin. These findings suggest that while the extracts were generally less potent than the antibiotic control, *F. vulgaris* and *A. rotundum* demonstrated promising inhibitory potential, particularly against *P. aeruginosa* and *K. pneumoniae*, respectively.

Table 1. Maximum inhibitory zone for extracts against tested bacterial strains at their highest tested concentrations.

Bacterial Strain	Inhibitory Zone Diameter (mm) at Highest Concentration of Ethanolic Extracts		
	A. rotundum (250 mg/mL)	*F. vulgaris* (250 mg/mL)	*D. aucheri* (166.5 mg/mL)
S. aureus	20	20	20
E. faecalis	22	16	55
P. aeruginosa	18	19	-
E. coli	20	18	22
K. pneumoniae	25	-	-

Table 2. Inhibitory zone diameters of *D. aucheri* ethanolic extract against bacterial strains. "–" indicates no inhibitory effect observed. GM: Gentamycin (positive control); DMSO: Dimethyl sulfoxide (negative control).

Concentration (mg/mL)	Inhibitory Zone Diameters (mm) for Tested Bacterial Strains				
	S. aureus	*E. faecalis*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
166.5	20	55	-	22	-
83.2	18	40	-	19	-
41.6	18	35	-	18	-
20.8	17	30	-	15	-
10.4	14	25	-	14	-
5.2	12	23	-	13	-
2.6	10	16	-	11	-
1.3	-	14	-	9	-
0.65	-	13	-	-	-
0.37	-	12	-	-	-
GM	27	28	18	28	28
DMSO	-	-	-	-	-

Table 3. Inhibitory zone diameters of *A. rotundum* ethanolic extract against bacterial strains. "–" indicates no inhibitory effect observed. GM: Gentamycin (positive control); DMSO: Dimethyl sulfoxide (negative control).

Concentration (mg/mL)	Inhibitory Zone Diameters (mm) for Tested Bacterial Strains				
	S. aureus	*E. faecalis*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
250	20	22	18	20	25
125	20	18	18	15	22
62.5	16	17	17	13	18
31.2	15	15	16	12	16
15.6	14	15	14	10	15
7.8	14	11	12	9	15
3.9	10	10	10	8	13
1.9	9	8	9	-	10
0.97	8	-	-	-	8
0.48	-	-	-	-	-
GM	27	28	18	28	28
DMSO	-	-	-	-	-

Table 4. Inhibitory zone diameters of various concentrations of *F. vulgaris* ethanolic extract against five standard bacterial strains using the well diffusion method. "–" indicates no inhibitory effect observed. GM: Gentamycin (positive control); DMSO: Dimethyl sulfoxide (negative control).

Concentration (mg/mL)	Inhibitory Zone Diameters (mm) for Tested Bacterial Strains				
	S. aureus	*E. faecalis*	*P. aeruginosa*	*E. coli*	*K. pneumoniae*
250	20	16	19	18	-
125	20	15	18	17	-
62.5	18	15	17	15	-
31.2	17	14	17	15	-
15.6	16	13	15	13	-
7.8	15	12	13	12	-
3.9	15	9	12	10	-
1.9	14	-	10	8	-
0.97	13	-	8	8	-
0.48	10	-	-	-	-
GM	27	28	18	28	28
DMSO	-	-	-	-	-

Table 5. Comparison of the maximum inhibitory effects of plant extracts with gentamycin (GM) against five bacterial strains. The table shows the closest inhibition zone values produced by each extract relative to gentamycin, along with the difference in diameter (mm). GM: Gentamycin (positive control).

Bacterial Strain	Gentamycin	Extract Closest in Effect	Inhibition Zone	Difference from GM
S. aureus	27	*A. rotundum* / *F. vulgaris* / *D. aucheri*	20	7
E. faecalis	28	*A. rotundum*	22	6
P. aeruginosa	18	*F. vulgaris*	19	1
E. coli	28	*D. aucheri*	22	6
K. pneumoniae	28	*A. rotundum*	25	3

Table 6. MIC values of three ethanolic plant extracts against five standard bacterial strains.

Bacteria	MIC Values of Ethanolic Extracts (mg/mL)		
	Flacaria vulgaris	*Allium rotundum*	*Dorema aucheri*
S. aureus	2.87	5.75	0.93
E. faecalis	5.75	23.0	0.46
P. aeruginosa	5.75	11.5	>15
E. coli	5.75	23.0	0.93
K. pneumoniae	>23	11.5	>15

The MIC values of the three ethanolic plant extracts against the five tested bacterial strains are summarized in Table 6. The lowest MIC observed in the entire study was 0.46 mg/mL, exhibited by *D. aucheri* against *E. faecalis*. Additionally, *D. aucheri* demonstrated strong inhibitory potential against *S. aureus* and *E. coli*, with MIC values of 0.93 mg/mL for both. In contrast, its activity against *P. aeruginosa* and *K. pneumoniae* was limited, with MIC values greater than 15 mg/mL, indicating relatively low efficacy at the tested concentrations. *F. vulgaris* showed the lowest MIC

against *S. aureus* (2.87 mg/mL), which was more potent than both *A. rotundum* and *D. aucheri* for this specific strain. However, its MIC values for *E. faecalis*, *P. aeruginosa*, and *E. coli* were uniformly 5.75 mg/mL, and no detectable activity was observed against *K. pneumoniae* (MIC > 23 mg/mL). *Allium rotundum* demonstrated moderate antibacterial activity, with MIC values of 5.75 mg/mL against *S. aureus*, 11.5 mg/mL against *P. aeruginosa* and *K. pneumoniae*, and 23 mg/mL against *E. faecalis* and *E. coli*, indicating reduced potency compared to the other extracts.

A Kruskal-Wallis test was conducted to evaluate the differences in MIC values among the three plant extracts across all bacterial strains. The analysis revealed a statistically significant difference ($H = 13.27$, $p = 0.0013$), indicating that the antibacterial potency varied significantly among the extracts. Pairwise comparisons (Dunn's test) confirmed that *D. aucheri* had significantly lower MIC values compared to *A. rotundum* ($p = 0.004$) and *F. vulgaris* ($p = 0.031$), supporting its superior antibacterial efficacy.

Discussion

A comparative evaluation of the two antibacterial assessment methods—well diffusion assay and MIC determination—revealed complementary but distinct patterns of effectiveness among the three Iranian medicinal plant extracts tested. While the well diffusion method primarily assessed the ability of the bioactive compounds to diffuse through agar and inhibit bacterial proliferation, the MIC test quantified the lowest concentration required to prevent visible microbial growth. Together, these methodologies enabled a more comprehensive evaluation of the antibacterial efficacy of *A. rotundum*, *F. vulgaris*, and *D. aucheri*. Notably, this investigation provided novel and promising data, as it is the first to examine the antibacterial properties of these three endemic Iranian species simultaneously against five clinically relevant bacterial strains with diverse structural and physiological characteristics. The findings not only highlight the distinctive antimicrobial spectra of each extract but also underscore their potential as bioactive agents.

Overall, *D. aucheri* emerged as the most potent extract, particularly in the MIC test, where it exhibited the lowest MIC value (0.46 mg/mL) against *E. faecalis*, and strong activity against *S. aureus* and *E. coli* (MIC = 0.93 mg/mL). Although it showed limited or no diffusion-based inhibition against *P. aeruginosa* and *K. pneumoniae* in the well method, its high potency at low concentrations in the MIC test against Gram-positive strains highlights its bacteriostatic potential, likely due to its active compounds requiring direct contact or specific solubility conditions. These findings align with those reported by Mianabadi et al., who demonstrated that methanolic extracts from various parts of *D. aucheri*—particularly the flower—exhibited notable antibacterial activity, especially against Gram-positive bacteria such as *Staphylococcus aureus* and *Bacillus cereus*. In their study, the flower extract showed the highest antimicrobial efficacy, with MIC values reaching as low as 10 mg/mL, and no activity was observed against *E. coli*, a result that contrasts with the present study's finding of a strong inhibitory effect against *E. coli* (MIC = 0.93 mg/mL) using ethanolic extract. This difference may stem from the use of different extraction solvents, plant collection sites (Yasuj vs. Kermanshah), or the part of the plant used (27).

These findings are generally consistent with those reported by Yazdi et al., who investigated both aqueous and ethanolic extracts of *D. aucheri* against a range of Gram-positive and Gram-negative pathogens. Their study revealed a broad spectrum of activity, with the ethanolic extract showing inhibition zones from 8 mm (*P. aeruginosa*) to 24 mm (*S. pyogenes*) and MIC values ranging between 2–64 mg/mL depending on the strain. Notably, *P. aeruginosa* exhibited the highest resistance, a pattern also observed in the present study. However, our findings revealed greater potency of the ethanolic extract, particularly against *E. faecalis* and *S. aureus*, with MICs <1 mg/mL, which may reflect regional

phytochemical variability or differences in extraction protocol and bacterial strains. Taken together, both studies reinforce the antibacterial promise of *D. aucheri*, particularly against Gram-positive bacteria, and support its further investigation as a plant-derived antimicrobial agent (28).

In contrast, *A. rotundum* showed the most consistent and broad-spectrum activity in the well diffusion assay. It was the only extract capable of inhibiting all five tested bacterial strains at 250 mg/mL, with the largest zone (25 mm) recorded against *K. pneumoniae*. However, its MIC values were generally higher (5.75–23 mg/mL), suggesting that although effective upon diffusion, higher concentrations were needed to achieve complete growth inhibition. This indicates a strong bactericidal effect through contact-based mechanisms, especially notable in *K. pneumoniae* and *P. aeruginosa*. The findings of the present study on *A. rotundum* are consistent with previous research by Dehpour et al., which demonstrated the antibacterial effects of the flower's essential oils—particularly against *K. pneumoniae* and *S. aureus*—with effective inhibition observed at low concentrations (<1/200 v/v). In that study, the plant was collected from northern Iran (Zagmarz region), whereas the current investigation focused on samples from the western province of Kermanshah. Despite differences in geographic origin and extraction method (essential oil vs. ethanolic extract), both studies highlighted the plant's broad-spectrum activity. This alignment reinforces the therapeutic promise of *A. rotundum* and suggests that its bioactivity may be attributed to conserved phytochemical constituents, such as organosulfur and phenolic compounds, across different habitats. The broader inhibition seen in the well diffusion assay in the present study complements the earlier essential oil findings, confirming *A. rotundum* as a valuable candidate for further development as a natural antimicrobial agent (29).

Based on the findings of Assadpour et al., who investigated the antioxidant and antihemolytic activities of *A. rotundum* collected from Mazandaran (northern Iran), the methanolic extract demonstrated significantly greater radical-scavenging capacity and metal-chelating ability compared to the essential oil. The extract exhibited an IC_{50} of 284 µg/mL in DPPH assay, showing superior antioxidant potential. Notably, the extract also showed strong nitric oxide and hydrogen peroxide scavenging abilities, indicating the presence of active polar phytochemicals with redox-modulating capabilities. When considered alongside the present study's broad-spectrum antibacterial activity of *A. rotundum*, especially its efficacy against *K. pneumoniae* and *P. aeruginosa*, these results further highlight the therapeutic promise of this species (30).

F. vulgaris demonstrated selective and moderate activity, with the most notable result being its inhibition of *S. aureus*—both in the well diffusion assay (20 mm zone) and MIC test (2.87 mg/mL), outperforming the other two extracts in this specific case. However, it failed to show any effect against *K. pneumoniae*, and its efficacy against *E. faecalis*, *P. aeruginosa*, and *E. coli* was relatively weak and uniform (MIC = 5.75 mg/mL). These findings align partially with previous work by Shafaghat, who reported antibacterial and antioxidant activities of *Falcaria vulgaris* essential oils collected from different regions in Iran (Ardabil and Khalkhal). The oils exhibited activity against several bacteria, with α-pinene, β-caryophyllene, limonene, and α-terpinyl acetate identified as the major bioactive compounds. Notably, the flower and leaf oils from both regions demonstrated moderate growth inhibition zones, confirming some antimicrobial potential (31). However, compared to the present study, where ethanolic extracts showed pronounced and selective inhibition of *S. aureus* with minimal impact on Gram-negative strains, the earlier study emphasized broader, though less potent, effects across test organisms. This suggests that both regional variation and extraction method (ethanolic vs. hydrodistilled oils) can significantly influence the antimicrobial profile of *F. vulgaris*.

These findings are further supported by the study of Kohsari et al., who synthesized silver

nanoparticles using *Falcaria vulgaris* aqueous extract and evaluated their antibacterial properties. Their results revealed strong inhibitory effects against *S. aureus* (ATCC 25923), with significantly lower MIC values (ranging from 0.535 to 0.001 µg/mL) compared to other bacteria tested. This corroborates the current study's observation of *F. vulgaris*'s selective potency against *S. aureus*. However, consistent with our results, their AgNP-Fv formulation exhibited minimal activity against *E. coli*, especially multidrug-resistant strains, and reduced effectiveness against *P. aeruginosa* (32). Collectively, these findings suggest that *F. vulgaris*—either as a crude extract or nanoparticle-mediated—exerts its antibacterial activity in a strain-specific manner, particularly effective against Gram-positive bacteria.

Our results are further corroborated by the work of Zangeneh et al., who synthesized gold nanoparticles using aqueous extracts of *F. vulgaris* leaves AuNPs@*F. vulgaris* and evaluated their biological activities. Their results showed that the nanoparticles exhibited broad-spectrum antibacterial effects, with MIC and MBC values ranging between 2-8 mg/mL and 2-16 mg/mL, respectively—comparable to or even surpassing standard antibiotics in potency ($p \leq 0.01$). Despite these promising outcomes, their MIC values against several bacterial strains fell within a range similar to or higher than those observed in the present study for crude extracts, suggesting that while nanoformulation enhances efficacy, the intrinsic antibacterial potential of *F. vulgaris* remains moderate and concentration-dependent (33). So, *F. vulgaris* exerts its primary antimicrobial impact selectively—most notably against *S. aureus*—and that its broader efficacy may benefit from formulation enhancements such as nanoparticle synthesis.

Our findings suggest that *D. aucheri* is the most suitable extract for targeting Gram-positive bacteria at low concentrations, particularly *E. faecalis*, due to its remarkably low MIC values. Meanwhile, *A. rotundum* appears to be the most versatile extract, demonstrating broad-spectrum inhibition via agar diffusion, especially against Gram-negative bacteria such as *K. pneumoniae* and *P. aeruginosa*. In contrast, *F. vulgaris* showed selective activity, with its strongest effect observed against *S. aureus* in both diffusion and MIC assays, though its limited efficacy against other strains reduces its broad therapeutic applicability.

The integration of both qualitative and quantitative methods provided robust and complementary evidence of antibacterial efficacy. The consistent inhibition of *E. faecalis* and *S. aureus*, coupled with variable responses from *K. pneumoniae* and *P. aeruginosa*, highlights both the spectrum and specificity of each plant extract. Importantly, ecological factors, particularly the geographic origin of the plant, play a major role in antimicrobial potency. Such regional phytochemical variability, underscores the significance of provenance in medicinal plant studies, and highlights the potential of leveraging Iran's vast geographic diversity to systematically compare the antibacterial performance of native plant species across different ecological zones (34, 35).

Conclusion

D. aucheri, *A. rotundum*, and *F. vulgaris* had promising antibacterial effects. Our findings not only validate the traditional applications, but also point to the therapeutic potential of these plants, particularly when sourced from Kermanshah Province. Future research should focus on phytochemical characterization, mechanism of action, and in vivo efficacy of these extracts, with specific emphasis on plants native to Kermanshah.

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Ethics approval and consent to participate

Not needed.

Conflict of interest

The authors declare that they have no conflict of interest.

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