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Antioxidant, antibacterial, and α-glucosidase inhibition potential of three *Allium* species (Amaryllidaceae) from Iran

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ABSTRACT

Introduction: Wild species of the genus *Allium* have high potential for use as medicine due to their essential secondary metabolites with antioxidant activity. This study explored the antioxidant, antibacterial, and α-glucosidase inhibition activities of three *Allium* species: *Allium tripedale, Allium hooshidaryae*, and *Allium stipitatum*.

Methods: The antioxidant potentials of the plant methanol extracts were evaluated using the ferric reducing antioxidant power (FRAP) and the 2,2-diphenyl-1picrylhydrazil (DPPH) radical scavenging test. Total phenolic content (TPC), total flavonoid content (TFC) and α-glucosidase inhibition were also evaluated. Antibacterial assessments were done employing disk diffusion and microdilution methods to determine inhibition zone and minimum inhibitory concentration (MIC), respectively against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Pseudomonas aeruginosa*.

Results: *Allium hooshidaryae* displayed high TPC (70.24 ± 0.0039 mg gallic acid equivalent/g extract), while *A. tripedale* had the highest TFC (87 ± 0.013 mg Quercetin equivalent/g extract). *A. hooshidaryae* showed superior antioxidant capacity (DPPH IC_{50}: 724.4 ± 0.31 µg/mL; FRAP: 36.87 mg ascorbic acid equivalent/g extract) and stronger α-glucosidase inhibition (IC_{50} = 2.59 mg/mL vs. 4.33 mg/mL for *A. tripedale* and 6.41 mg/mL for *A. stipitatum*). Qualitative tests confirmed phenolic, flavonoid, and glycoside compounds in all three species. *A. stipitatum* uniquely contained saponin and tannin. *A. hooshidaryae* and *A. stipitatum* inhibited the bacterial strains effectively, especially at the higher concentration (400 µg/mL). *A. stipitatum* showed inhibition against all strains, particularly against *S. aureus* (MIC: 12.5 µg/mL).

Conclusion: This study highlights the antidiabetic and antibacterial potential of three *Allium* species, emphasizing their values as rich sources of bioactive compounds.

Implication for health policy/practice/research/medical education:
The leaf extract of *Allium hooshidaryae* was found to contain phytochemicals with notable antioxidant and antidiabetic properties. Additionally, *Allium stipitatum* exhibited strong antibacterial effects. Therefore, these plants might be promising natural remedies for diabetes mellitus and may be valuable in the search for alternative treatments for infectious diseases.

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Introduction

Various *Allium* species constitute a significant member of the Amaryllidaceae family, representing arguably the most extensive genus within the petaloid monocotyledons, encompassing approximately 900 distinct species (1). The genus is markedly characterized by its bulbous structures, typically enveloped in membranous (occasionally transitioning to fibrous) tunics. This genus is noteworthy for its production of considerable quantities of cysteine sulphoxides, responsible for the distinctive odor and taste associated with these plants. The *Allium* genus boasts its principal center of diversity in regions including the Eastern Mediterranean, Southwest, and Central Asia (2). Various wild *Allium* species are harvested by local

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populations for consumption, serving as food, medicinal plants, or ornamental additions. These wild variants hold substantial potential for incorporating daily human nutritional and medicinal values (1). Specifically, within the Kurdistan province of Iran, *A. hooshidaryae*, *A. stipitatum*, and *A. tripedale* are favored as traditional food sources during the spring season. Notably, *A. hooshidaryae* is endemic to Kurdistan; *A. tripedale* is a native species predominantly found in Northwestern Iran, and *A. stipitatum* thrives across the Zagros Mountains, enjoying widespread distribution within the Iranian region.

Species within the *Allium* genus are abundant reservoirs of crucial secondary metabolites, encompassing steroidal saponins and sapogenins, flavonoids, and organosulfur compounds. A plethora of pharmacologically potent compounds have been successfully isolated from various *Allium* species (3). Phenylpropanoid glycosides (PPGs), highly regarded natural phenolic compounds, are significant secondary metabolites in various plant species. Studies have shown that PPGs possess a wide range of pharmacological activities (4). These phenolic compounds are notable for their strong antioxidant, anti-inflammatory, antimicrobial, and anti-carcinogenic properties effective in preventing chronic diseases linked to oxidative stress. The phenolic contents in plants are influenced by various environmental factors, such as soil type, rainfall, sunlight, pathogen presence, and harvesting methods (5).

The enzyme α-glucosidase plays a crucial role in breaking down α-glycosidic bonds in oligosaccharides and disaccharides into monosaccharides during digestion, leading to higher blood glucose levels. Inhibiting α-glucosidase is a key strategy in managing postprandial hyperglycemia. Apart from the inhibition of alpha-glucosidase enzyme, most plants are also rich in natural antioxidants, which help counteract the harmful effects of reactive oxygen species (ROS), thereby lowering the risk of developing and progressing type 2 diabetes mellitus (T2DM) (6).

The *Allium* genus exhibits anti-diabetic properties due to compounds such as S-benzyl-cysteine, diosgenin, polysulfanes, fisetin, allicin (diallyl thiosulfinate), tuberoside M, S-allyl mercapto cysteine, thiosulfinates, S-propargyl-L-cysteine, Ace-AMP1, and quercetin. In Iranian traditional medicine, these plants are used to treat various metabolic disorders, including diabetes (7).

Extensive empirical research has unveiled that *Allium* species and their corresponding sulfide constituents exhibit potent antimicrobial properties. Notably, these entities manifest a pronounced antifungal activity, surpassing their antibacterial effects (8-12). The current study examines the phytochemistry, antibacterial properties, and α-glucosidase inhibition potential inherent to selected wild edible *Allium* species, consumed by local people, namely *A. tripedale*, *A. hooshidaryae*, and *A. striatum*.

Materials and Methods

Sample collection

Three species of *Allium* genus including *Allium tripedale* Trautv. *Allium hooshidaryae* Mashayekhi, Zarre & R.M. and *Allium stipitatum* Regel. were collected from the Saral area of Kurdistan Province, Iran. After taxonomic identification of the collected plants, their specimens were deposited in the herbarium unit of the Biological Science Department, University of Kurdistan, Iran (Table 1). The collected samples were dried at room temperature (22 ˚C) under low light conditions for one week. The aerial parts of the plants were finely ground and soaked in methanol as a solvent (10 mL/g of plant). The samples were placed on a shaker in dark conditions for 72 hours. After filtration using Whatman paper (42, ash), the extracts were dried by rotary evaporation equipped with a vacuum pump and stored at -20 ˚C for future use.

Preliminary phytochemical screening

For each of the phytoconstituents, one milligram of the dried extract was dissolved in 10 mL of methanol and used as a stock solution for the following tests:

Test for alkaloids (Wagner's test)

To prepare Wagner's reagent, 2 g of iodine and 6 g of potassium iodide were dissolved in 100 mL water. One or 2 mL of the stock solution was added to the Wagner's reagent. In the presence of soluble alkaloids, the color of the solution turned red (13).

Test for tannins (Ferric chloride test)

Two mL of the stock solution was added to ferric chloride (10%). In the presence of tannins, the color of the solution turned dark bluish-green (14).

Test for saponins (Frothing test)

Two milliliters of stock solution was added to 5 mL of water. The tube containing the solution was then shaken for 10 seconds and held for 30 minutes. The formation of a stable foam at the top of the tube indicated the presence of saponins (13).

Table 1. Specifications of the studied plants

Plant family	Scientific name	Local name	Latitude	Longitude	Voucher specimen
Amaryllidaceae	*Allium tripedale*	Pichek	35̊39′49″ N	47̊10′01″E	UOK-215
	Allium hooshidaryae	Psel- Loosha	35̊44′20″ N	46̊40′41″E	UOK-211
	Allium stipitatum	Mu-sir	35̊24′15″ N	46̊58′37″E	UOK-204

Test for flavonoid (Alkaline reagent test)

NaOH (1M) was added dropwise to 1 mL of the stock solution. The color of the solution turned yellow. Changing the color to colorless after adding a drop of diluted HCL indicated the presence of flavonoids (13).

Test for sterols (Salkowski's test)

Two millilitres of chloroform was added to 1 mL of the stock solution. Concentrated H2SO4 was then slowly added. The formation of a brown color between the solution and chloroform indicated the presence of sterols (13).

Test for cardiac glycoside (Keller-Kiliani test)

To 2 mL of the stock solution, 1 mL of pure glacial acetic acid and one drop of FeCl3 (5%) were added. Then, concentrated H2SO4 was added to the solution. The formation of a brown color in the middle and a blue-green color in the upper layer indicated the presence of glycosides (13).

Test for Phlobatannins (HCl test)

To 1 or 2 mL of stock solution, 1 mL of HCL (1%) was added and boiled. The formation of a red precipitate indicated the presence of phlobatannins (14).

Assessment of antioxidant capacity using ferric reducing antioxidant power (FRAP) and 2,2-diphenyl-1picrylhydrazil (DPPH) radical scavenging test

The reducing power of the examined extracts was assessed utilizing the methodology delineated by Yen and Chen (15). The extracts were prepared at diverse concentrations, namely 50, 100, 200, 400, 600, and 800 µg/mL. To each 2.5 mL aliquot of these varying concentrations, 2.5 mL of 0.2 M phosphate buffer and 2.5 mL of 1% potassium ferricyanide were subsequently added. The ensuing reaction mixtures were then incubated at a temperature of 45 °C for 30 minutes. To terminate the reactions, 2.5 mL of 10% trichloroacetic acid was introduced to each mixture. This was followed by a centrifugation process conducted at 3000 rpm for a 15-minute interval, post which 2.5 mL of the resultant supernatant was carefully extracted and combined with 0.5 mL of 1% ferric chloride. Absorbance readings of the final mixtures were recorded at a wavelength of 700 nm. For comparison purposes, ascorbic acid was employed as a positive control at concentrations analogous to those of the extracts under investigation. Data accrued from this process facilitated the construction of the standard curve.

To evaluate the DPPH radical scavenging activity inherent to the sample, a solution constituting 1 mL of 0.1 $mmol.L^{-1}$ DPPH dissolved in methanol was amalgamated with 3.0 mL of the extract prepared at varying concentrations (62.5, 125, 250, 500, and 1000 µg/mL) (16). After the combination, the mixture was incubated in an environment devoid of light at ambient room temperature for 15 minutes. Upon completion of the incubation period, the absorbance of the solution was ascertained at 517 nm utilizing a spectrophotometer, as delineated in reference (17). The scavenging activity was quantitatively expressed as the inhibition percentage (I %), calculated employing the equation: $I\% = [(OD_{control} - OD_{sample}) / OD_{control}] \times 100$. In this equation, OD sample denotes the absorbance of the solution containing the extract, whilst OD control represents the absorbance of the control solution. A heightened value of I% is indicative of the potent antioxidant activity manifested through its efficacious neutralization of DPPH radicals.

Assessment of total phenolic content (TPC)

TPC was determined using the Folin-Ciocalteu method (18). In this procedure, 1 mg of the extract was dissolved in 10 mL of methanol. Then, 0.5 mL of the solution and 0.5 mL of Folin-Ciocalteu reagent were mixed and stirred for two minutes. Next, 10 mL of 7% sodium carbonate solution was added, and the mixture was incubated at 45 °C for one hour. Absorbance was measured at 765 nm. A standard curve was generated using gallic acid solutions at concentrations ranging from 10 to 100 µg/mL. The method relied on the reduction of Folin-Ciocalteu reagent by phenolic compounds in the extracts, resulting in a blue complex.

Assessment of total flavonoid content (TFC)

The TFC in plant extracts was quantified using the aluminium chloride colorimetric method (19). The extract solutions of 0.1 mg/mL were prepared, with 0.5 mL of each mixed with 1.5 mL of methanol. To this mixture, 0.1 mL of 10% aluminum chloride, 0.1 mL of 1M potassium acetate, and 2.8 mL of distilled water were added. After a 30-minute incubation at room temperature, absorbance was measured at 415 nm. A standard curve was created using quercetin, and the results were expressed in quercetin equivalents per gram of extract. The procedure was repeated thrice, and average values were reported. The method identified flavonoids present in the extracts through the formation of an acidic complex between aluminum chloride and flavonoid hydroxyl groups.

α-Glucosidase inhibition assay

The α-glucosidase inhibition assay for the extract and its fractions was performed following a slightly modified standard protocol (20). A 96-well plate was used to mix 50 µL of phosphate buffer (100 mM, pH 6.8), 10 µL of alpha-glucosidase (1 U/mL), and 20 µL of different concentrations of the extract (0.5-2.5 mg/mL). This mixture was pre-incubated at 37 °C for 15 minutes. Subsequently, 20 µL of p-nitro phenol glucopyranoside (pNPG) (5 mM) was added as the substrate, followed by another incubation at 37 °C for 20 minutes. The reaction was halted by adding 50 µL of $Na_2 CO_3$ (0.1 M). The absorbance of the liberated p-nitrophenol was read at 405 nm using a multiplate

reader. Acarbose, in concentrations ranging from 0.03 to 2 mg/mL, served as a reference standard. A control without the test substance was prepared simultaneously, and each experiment was conducted in triplicate. The inhibitory results were expressed as a percentage using the following formula:

Inhibitory activity (%) = (1 − As/Ac) ×100

where *As* represents the absorbance with the test substance and *Ac* represents the absorbance of the control.

Antibacterial assay

The antibacterial activity of plant extracts was evaluated against four clinical isolates bacterial strains: two gram-negative bacteria (*Pseudomonas aeruginosa* and *Escherichia coli*) and two gram-positive bacteria (*Staphylococcus aureus* and *Bacillus subtilis*). The nutrient agar medium facilitated the passage, while the Mueller-Hinton medium was employed for the bacterial growth. Antibiotic discs, namely chloramphenicol, nitrofurantoin, vancomycin, amikacin, ciprofloxacin, meropenem, and imipenem, served as comparative positive controls in the assay.

Using the disk diffusion method, pure microorganism colonies were suspended in distilled water to achieve 0.5 McFarland turbidity (1.5×10^8 CFU/mL). Each strain (100 µL) was inoculated onto Mueller-Hinton medium. Sterile 6 mm paper discs were soaked into 100 µL of plant extracts at concentrations of 20, 50, 200, and 400 µg/mL and placed on inoculated agar plates. Plates were incubated at 37 °C for 24 hours. Tests were conducted in triplicate, with antibacterial activity assessed by measuring inhibition zones (including the 6 mm disc). Antibiotic discs were used as positive growth control, and 10% DMSO served as the negative growth control.

The minimum inhibitory concentration (MIC) was determined using the tube microdilution method. The dilutions of 12.5, 25, 50, 100, 200, and 400 µg/mL were prepared from the methanolic extract in Mueller Hinton broth. Each dilution received a bacterial suspension of 1.5×10^8 CFU/mL. Tubes were set as positive controls (with bacteria but without extract) and negative controls (without bacteria). After incubation at 37 °C for 24 hours, the tubes were examined for turbidity to indicate bacterial growth. The last non-turbid tube was identified as MIC. For minimum bactericidal concentration (MBC), 100 µL from the three tubes preceding the MIC tube were cultured. The lowest extract concentration preventing bacterial growth was reported as the MBC.

Statistical analysis

The dataset, consisting of triplicate measurements, was subjected to statistical analysis using analysis of variance (ANOVA), and the results were presented as mean values ± standard error of the mean (SEM). Two-way ANOVA, complemented by Tukey's multiple comparison tests (data not shown), was employed for data analysis through SPSS version 16.0 (SPSS Inc., USA), considering a P value < 0.05 as the threshold for statistical significance. The standard curve was plotted to obtain the line equation using Excel software.

Results

The antioxidant capacity of the plants under study was assessed employing the FRAP assay and the DPPH scavenging assay. Within the framework of the FRAP assay, the regenerative powers of the methanolic extract derived from the plants under scrutiny were appraised, contingent on its ability to reduce the Fe^{3+}/ferricyanide complex to its ferrous counterpart. The mean reduction percentage plot, as illustrated in Figure 1A delineated the reduction percentage for the *Allium* species studied, juxtaposed with those of ascorbic acid. Upon comparison of the reducing power, *A. hooshidaryae* manifested the most potent antioxidant activity across almost all concentration levels,

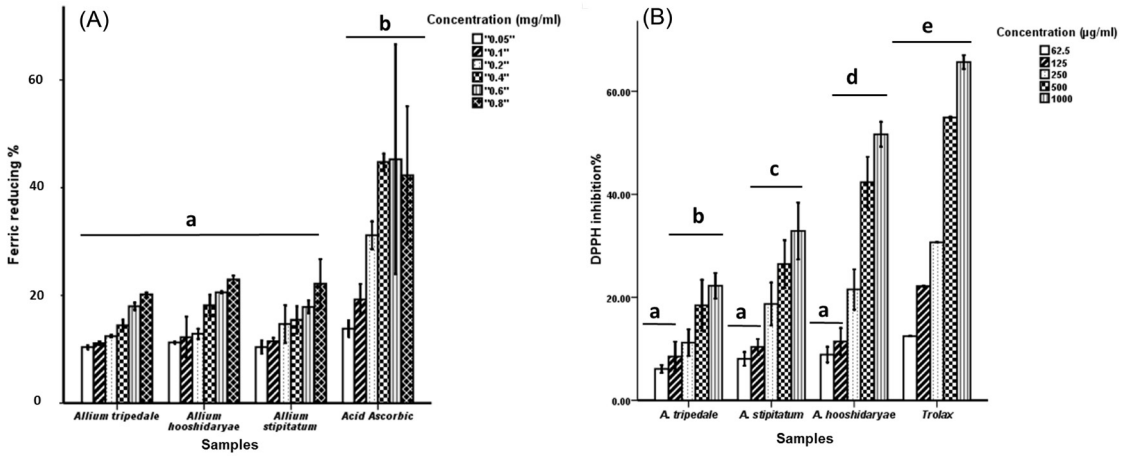

Figure 1. Antioxidant activities of the methanol extracts of *Allium* species measured by ferric reducing (A) and 2,2-diphenyl-1-picrylhydrazyl (DPPH) (B) methods (mean ± SEM; n = 3). Bars with different letters indicate significant differences between groups (P < 0.05, according to the Tukey test).

with the sole exception at the 0.2 mg/mL concentration. However, variance analysis elucidated that the differences in ferric-reducing power among the three *Allium* species studied were not statistically significant ($P > 0.05$).

DPPH is a stable free radical. Its interaction with an antioxidant leads to electron pairing within the DPPH molecule, causing the solution to decolorize. The extent of this decolorization, or scavenging activity, directly correlates with the number of electrons the antioxidant donates to DPPH. The DPPH scavenging activity of studied plant extracts exhibited a concentration-dependent relationship (Figure 1B). As extract concentration increased, so did DPPH radical scavenging capacity. The positive control, Trolox, demonstrated significant scavenging activity with an IC_{50} value of 649.21±0.03 $\mu g.mL^{-1}$.

Notably, the methanol extract of *A. hooshidaryae* had an IC_{50} value of 724.4 ± 0.3 $\mu g.mL^{-1}$ (Table 2), showcasing superior scavenging activity compared to two other species that couldn't achieve 50% inhibition even at 1000 $\mu g.mL^{-1}$. Thus, *A. hooshidaryae* emerges as the most effective DPPH radical scavenger among the examined species. Variance analysis indicated that there was a significant difference among all concentrations of plant extracts, except for the 62.5 and 125 µg/mL concentrations. Additionally, significant differences were observed between the plants and Trolax at a $P < 0.05$.

Figure 2 presents the α-glucosidase inhibitory activities of methanolic extracts from the three studied Allium species. The results showed that the methanolic extracts effectively inhibited α-glucosidase. Among the species, *A. hooshidaryae* exhibited the highest inhibitory activity (IC_{50} = 2.59 mg/mL), followed by *A. tripedale* (IC_{50} = 4.33 mg/mL) and *A. stipitatum* (IC_{50} = 6.41 mg/mL). The percentage of α-glucosidase inhibitory activity was significantly different among the three species ($P < 0.05$).

The susceptibility and resistance of four gram-positive and gram-negative bacteria, including *P. aeruginosa*, *E. coli*, *S. aureus,* and *B. subtilis* were evaluated in different concentrations of *Allium* species extract in three replicates (Table 3). The zone of growth inhibition of the studied bacteria in the presence of some antibiotics and solvent (DMSO 10%) represented as mean ± standard deviation

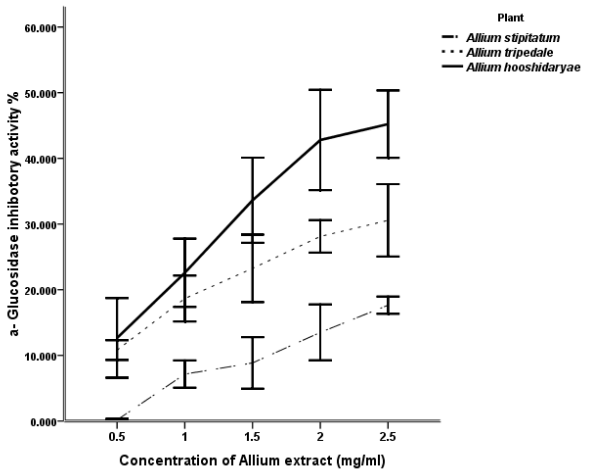

Figure 2. α-Glucosidase inhibitory activities of the methanolic extracts of the studied Allium species. Results represent mean± SD of three trials (n= 3). The inhibitory percentage significantly differed among the three studied species ($P < 0.05$).

(Table 4). *A. tripedale*, in different concentrations, inhibited the growth of *S. aureus* and *B. subtilis* at 400 µg extract/mL, with growth inhibition zones of 18 ± 0.02 mm and 16 ± 0.62 mm in diameter, respectively. However, it did not affect the other studied bacteria strains. Similar results were observed for *A. hooshidaryae* (*S. aureus*: 16 ± 0.024 mm and *B. subtilis:* 16 ± 0.03 mm) However, 400 µg/mL of *A. hooshidaryae* made 12± 0.205 and 13 ± 0.34 mm of inhibition zone for *P. aeruginosa* and *E. coli,* respectively.

Allium stipitatum demonstrated the strongest ability to inhibit the growth of *S. aureus* at all concentrations and *E. coli* at 400 µg/mL of extract (14 ± 0.04 mm). The effect of *A. stipitatum* extract was similar to vancomycin, nitrofurantoin, and chloramphenicol antibiotic discs, and the result of the analysis of variance confirmed that the mean effectiveness of this species compared to the mentioned antibiotics was not significant (0.05). Besides, even at the lowest used concentration (20 µg/mL), this species made a small inhibition zone (5 ± 0.202 mm) on *P. aeruginosa*, the strain completely resistant to all antibiotics used as positive controls.

Analysis of variance showed that all studied factors, including plant species, concentrations of plant extracts,

Table 2. Total phenolic and flavonoid contents and preliminary screening of phytochemicals in Allium studied species extract

Plant family	Scientific name	Phenolics (mg gallic acid per g extract)	Flavonoids (mg quercetin per g extract)	IC_{50} of DPPH inhibition (µg. mL^{-1})	IC_{50} of AG inhibitory mg/mL	Mg ascorbic acid/g extract	Chemical constituents*					
							A	Sa	Phl	T	St	G
Amaryllidaceae	*A. tripedale*	24.38 ± 0. 001	87 ± 0.013	1275±0.18	4.33± 0.41	32.21± 0.12	-	-	-	-	-	+
	A. hooshidaryae	70.24 ± 0.004	29.18 ± 0.001	724.4 ± 0.31	2.59± 0.021	38.87± 0.02	-	-	-	-	+	+
	A. stipitatum	46.07± 0.019	56.45 ± 0.034	1141±0.03	6.41± 0.071	33.4± 0.041	-	+	-	+	+	+

DPPH: 2,2-diphenyl-1-picrylhydrazyl; AG: Alpha-glucosidase; A: Alkaloids; Sa: Saponin; Phl: Phlobatannin; T: Tannins; St: Sterols; G: Glycosides. Data are presented as Mean ± SEM (n = 3; $P < 0.05$). *Presence (+) or absence (-) of chemical compounds.

Table 3. Inhibition zone diameters of leaf extracts from the studied *Allium* species against the tested microorganisms

Bacteria	Plant extract	20 µg/mL	50 µg/mL	200 µg/mL	400 µg/mL
Pseudomonas aeruginosa	*Allium tripedale*	0	0	0	0
Escherichia coli		0	0	0	0
Staphylococcus aureus		0	0	0	18 ± 0.02
Bacillus subtilis		0	0	0	16 ± 0.62
Pseudomonas aeruginosa	*Allium hooshidaryae*	0	0	0	12 ± 0.205
Escherichia coli		0	0	0	13 ± 0.34
Staphylococcus aureus		0	0	0	16 ± 0.024
Bacillus subtilis		0	0	0	16 ± 0.03
Pseudomonas aeruginosa	*Allium stipitatum*	5 ± 0.202	6 ± 0.12	8 ± 0.601	10 ± 0.27
Escherichia coli		5 ± 0.57	7 ± 0.17	10 ± 0.78	14 ± 0.04
Staphylococcus aureus		10 ± 0.01	15 ± 0.062	17 ± 0.032	19 ± 0.026
Bacillus subtilis		9 ± 0.07	13 ± 1.03	15 ± 0.42	17 ± 0.32

Table 4. Bacterial growth inhibition zone in the presence of antibiotics used as positive control and DMSO 10% as negative control (mean ± SD) (mm)

Bacteria/Antibiotic	CH	NI	AM	VA	CI	ME	IM	DMSO 10%
Pseudomonas aeruginosa	0	0	0	0	0	0	0	0
Escherichia coli	18 ± 2.32	0	17 ± 1.54	0	0	0	0	0
Staphylococcus aureus	16 ± 1.02	16.6 ± 0.45	0	19± 0.52	0	0	0	0
Bacillus subtilis	18± 0.06	18± 1.09	16±0.23	16±0.46	16± 0.23	0	19± 0.31	0

DMSO: Dimethyl sulfoxide; CH: Chloramphenicol; NI: Nitrofurantoin; AM: Amikacin; VA: Vancomycin; CI: Ciprofloxacin; ME: Meropenem; IM: Imipenem. Antibiotic disk content = 20 µg.disk^{-1}

strains of bacteria, and their interactions had significant differences in inhibiting bacterial growth at a significant level of 0.05 (data not shown). The antibacterial effects of the studied plants showed an elevating trend with increasing concentrations of plant extracts. In addition, *A. stipitatum* had the greatest effect on bacterial growth inhibition.

The results of the tube dilution method (Table 5) showed that the extracts of the studied plants had different inhibitory effects in the liquid medium. The highest inhibitory effect in the lowest concentration was related to *A. stipitatum* on *S. aureus* (MIC 12.5 µg.mL^{-1}). *Allium hooshidaryae* and *A. tripedale* both exhibited the same MIC of 50 µg/mL against *S. aureus* and *B. subtilis*. It should be noted that the effect of growth inhibition (MIC) of all three plant species against *P. aeruginosa* was weak (100 µg.mL^{-1}).

The phytochemical compositions of the selected *Allium* species were screened (Table 2). According to the data presented, none of the analyzed species exhibited

Table 5. MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration) levels of plant extracts on the studied bacteria strains

Plant species	Bacteria	MIC (µg.mL^{-1})	MBC (µg.mL^{-1})
Allium tripedale	*Staphylococcus aureus*	50	100
Allium hooshidaryae		50	100
Allium stipitatum		12.5	25
Allium tripedale	*Escherichia coli*	-	-
Allium hooshidaryae		50	100
Allium stipitatum		50	100
Allium tripedale	*Pseudomonas aeruginosa*	-	-
Allium hooshidaryae		100	200
Allium stipitatum		100	200
Allium tripedale	*Bacillus subtilis*	50	100
Allium hooshidaryae		50	100
Allium stipitatum		25	50

detectable levels of phlobatannins and alkaloids, suggesting that these compounds were either absent or present in undetectable amounts. The universal presence of glycoside and flavonoid compounds was noteworthy across all studied species. Furthermore, only *A. stipitatum* tested positive for saponins. Similarly, tannins were identified exclusively in *A. stipitatum*. The TPC found in the methanolic extracts of the *Allium* species under investigation (Table 2). In the process of calculating the concentrations of phenolic compounds within the extracts, a standard curve derived from gallic acid was employed, as depicted in Figure 3A. The results revealed a conspicuous variation in phenolic content among the studied *Allium* species. Notably, *A. hooshidaryae* exhibited the highest concentration of phenolic compounds, with a content of 70.24±0.0039 mg of gallic acid equivalents per gram of extract (mg GA/g extract). Conversely, *A. tripedale* displayed the lowest phenolic content, measured at 24.38±0.001 mg GA/g extract.

The flavonoid content was ascertained using the calibration curve of quercetin ($y = 0.0011x + 0.074$, $R^2 = 0.96$) (Figure 3B). Among the studied plants, *A. tripedale* had the highest content of flavonoids, registering at 87±0.013 mg QE/g of the dry material. Conversely, *A. hooshidaryae* exhibited the lowest flavonoid content, amounting to 29.18±0.001 mg of quercetin per gram of extract (Table 2). The ANOVA indicates significant differences in phenol and flavonoid content among the species studied ($P<0.05$).

Discussion

According to the obtained results, the antioxidant properties of the examined plant species were confirmed by Ferric reducing and DPPH radical scavenging methods. The data analysis outcomes showed the dependency of antiradical properties of extracts on concentration. The increased antiradical properties of the studied *Allium* species can be attributed to the presence of phenolic or flavonoid compounds. In the present study, *A. hooshidaryae* had the highest antioxidant capacity and scavenging properties of free radicals, probably due to the higher phenolic content observed in the plant (70.24 mg GA/g extract) (21).

There is extensive literature on the antioxidant and antibacterial properties of cultivated *Allium* species such as garlic and onion (22-24) but research on wild species has been limited. *Allium sativum* has been reported to possess significant antioxidant and antifungal properties. It increases total thiols and reduces serum total oxidative status, nitric oxide production, and malondialdehyde levels. The plant exhibits considerable antioxidant effects in turpentine-induced inflammation and has antifungal impacts on *Rhodotorula mucilaginosa* and *Meyerozyma guilliermondii* (25). These findings are consistent with the present study highlighting strong antioxidant and antimicrobial activities for *Allium* species. These researchers also found that the method of extraction influenced the antioxidant activity and the extraction with 70% ethanol gives the highest TPC and reduction capacity values (26).

The antibacterial and antibiofilm activities of hexane and dichloromethane extracts of *A. stipitatum* were assessed. Results demonstrated the possible therapeutic use of this species to hinder the growth of clinically significant gram-positive and gram-negative biofilms suggesting the need for further research into the potential of *A. stipitatum* bulbs in combating biofilm-related drug resistance. The permeability of *Allium*-inhibitory substances may be affected by the cell wall, cell membrane lipids, and cell membrane polysaccharides of bacterial strains (27). However, the gram-negative bacteria were less susceptible to diallyl trisulfide, dimethyl trisulfide, and garlic extract, while the Gram-positive bacteria were more sensitive (9-28). Garlic extracts are effective against a wide range of multidrug-resistant saprophytic and pathogenic bacteria (29).

The hydromethanolic extract of *A. hooshidaryae* showed higher anti-radical and cytotoxic activities rather than that of the obtained essential oil. The essential oil exhibited anti-*S. aureus* and anti-*E. coli* activities

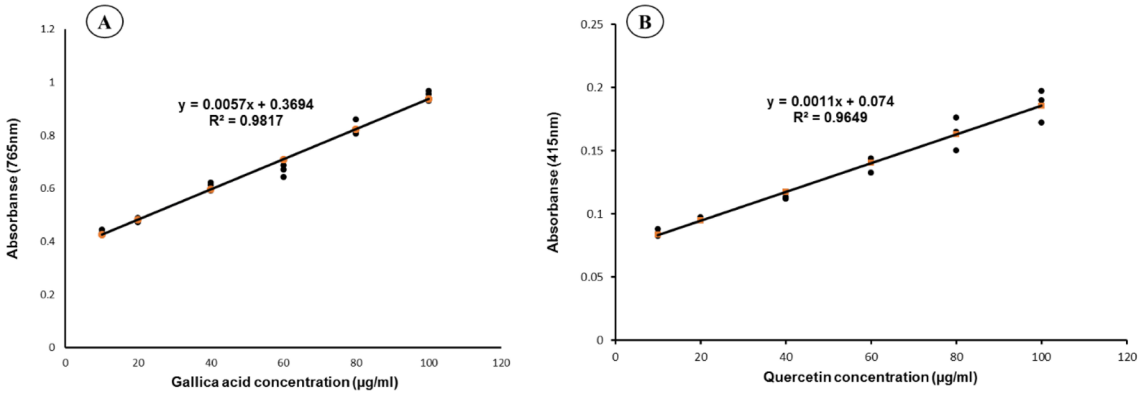

Figure 3. Calibration curves of gallic acid (A) for subsequent determination of phenolic and quercetin (B) for subsequent determination of flavonoid contents in the studied species.

approximately the same as chloramphenicol (30). *Allium tripedale* extract had a considerable inhibitory effect against various *Candida* species (31). The presence of flavonoids and sesquiterpenoids, diphenylamine, and iron as microelements was reported in *A. tripedale*, and the methanol extract of *Nectaroscordum tripedale* leaves had significant antibacterial activities (31,32).

The inhibitory activity of the studied *Allium* species leaves on the enzyme α-glucosidase is likely associated with saponins, tannins, and flavonoids detected in the methanolic extracts (33). In the present study, *A. hooshidaryae* exhibited significant α-glucosidase inhibitory activity (IC_{50} 2.59 mg/mL), which could be associated with the relatively high DPPH and FRAP antioxidant activity (DPPH IC_{50}: 724.4 ± 0.31 µg/mL; FRAP: 36.87 mg ascorbic Acid/g extract). This is the first report of α-glucosidase inhibitory activity related to the studied species, and the results were consistent with the findings of Choi et al, who reported significant anti-diabetic effects of *A. hookeri* ethanolic extract (34). Compared to *A. cepa* (peeled bulbs and bulbs), the extracts of *A. hooshidaryae* exhibited stronger AG inhibitory activity with IC_{50} values of 0.035 ± 0.01 and 0.052 ± 0.01 mg/mL. AG inhibitory activity of *A. sativum* bulbs extracts (IC_{50}: 2.51 ± 0.5 mg/mL) was almost the same as *A. hooshidaryae* leaf extract (35).

Regarding the scientific relationship, α-glucosidase inhibition and antioxidant activity are independent activities, though they may synergistically be useful in the control of diabetes. The potential link between these properties may contribute to the overall therapeutic effect, particularly in managing oxidative stress and hyperglycemia in diabetes.

Phytochemical analysis showed that the methanolic leaf extracts of three *Allium* species contained glycosides. Tannins and saponins were present only in *A. stipitatum*, while sterols were identified in *A. hooshidaryae* and *A. stipitatum*. In this study, saponins were discovered exclusively in the *A. stipitatum* extract. Saponins have a broad range of bioactivities, including antidiabetic, antiallergic, anti-inflammatory, antinociceptive, and anticancer properties (36). The qualitative phytochemical screening results suggest that the methanolic leaf extracts of the studied *Allium* species could be used as natural pharmacological agents. Flavonoids, which are secondary metabolites, are linked to numerous medicinal benefits, including antioxidant, anti-aging, antiviral, and anti-inflammatory effects (37).

In this experiment, the total flavonoid content in the *Allium* species ranged from 29.18 to 87 mg quercetin/g dry extract (Table 2). This flavonoid content was higher than that in the leaf extracts of *A. cepa* (0.06–1.92 mg/g DW) (38). The comparison study on total phenols of some *Allium* species, including garlic (*A. sativum* L.), chives (*A. schoenoprasum* L.), ramson (*A. ursinum* L.), and red, yellow, and white onion (*A. cepa* L.), indicated that phenol contents of studied *Allium* species were in the range of 444.3 to 1591 $mg.kg^{-1}$, with *A. schoenoprasum* and *A. cepa* showing the highest and lowest amounts, respectively (38). The phenol content observed in the species under study was greater than that found in cultivated species.

Phenolic content in the ethanol extracts of different populations of *A. hirtifolium* ranged from 34 to 44 mg gallic acid/g extract (39). *Allium hirtifolium* is phenotypically similar to *A. stipitatum*, despite having independent evolutionary lineages (40). Additionally, the extracts of *A. hirtifolium* demonstrated moderate to good inhibitory activities (MICs 0.062 to 0.250 mg/mL) against *Bacillus cereus* (39).

Conclusion

This study aimed to explore and compare the antioxidant, α-glucosidase inhibitory, and antibacterial activities of methanolic extracts from three *Allium* species. The antioxidant assessment demonstrated that *A. hooshidaryae* possessed the most potent antioxidant activity, especially in the DPPH assay. However, in the FRAP assay, the differences in ferric reducing power among the species were not statistically significant. The methanolic extracts of all three *Allium* species were effective in inhibiting α-glucosidase, with *A. hooshidaryae* showing the highest inhibitory activity. The antibacterial effects varied significantly among the species. *Allium stipitatum* showed the most substantial inhibitory effect against a range of bacteria, including *S. aureus*, *B. subtilis*, and *E. coli*. It was particularly effective against *S. aureus*, with a MIC of 12.5 µg/mL. *Allium hooshidaryae* and *A. tripedale* also demonstrated notable antibacterial properties, though to a lesser extent. Overall, these findings support the potential of wild *Allium* species, particularly *A. hooshidaryae* and *A. stipitatum*, as valuable sources of natural antioxidant, antidiabetic, and antimicrobial agents. Further research into their specific bioactive compounds and their mechanisms of action may lead to the development of novel therapeutic agents.

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Authors' contributions

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Formal analysis: Shahla Hosseini.

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Methodology: Shahla Hosseini, Mohammad Ali Zarei and Musa Moetasam Zorab.

Project administration: Shahla Hosseini.
Resources: Shahla Hosseini, Mohammad Ali Zarei and Musa Moetasam Zorab.
Software: Shahla Hosseini.
Supervision: Shahla Hosseini and Mohammad Ali Zarei.
Validation: Shahla Hosseini, Mohammad Ali Zarei and Musa Moetasam Zorab.
Visualization: Shahla Hosseini, Mohammad Ali Zarei and Musa Moetasam Zorab.
Writing–original draft: Shahla Hosseini.
Writing–review & editing: Shahla Hosseini, Mohammad Ali Zarei and Musa Moetasam Zorab.

Conflict of interests

The authors declare that there is no conflict of interest.

Data availability statement

All data are presented in this manuscript; however, further details can be requested from the authors.

Ethical considerations

This study did not include the use of animal or human models. The authors meticulously followed ethical standards, ensuring the absence of plagiarism, misconduct, data fabrication, falsification, duplicate publication, submission, or redundancy.

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