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Antioxidant and antimicrobial activity of extracts from selected *Allium* species

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ABSTRACT *Allium* vegetables are widely consumed and represent important dietary sources of organosulfur compounds and phenolics with potential antioxidant and antimicrobial relevance. In the present study, hydroethanolic extracts prepared from onion (*Allium cepa*; young/spring, summer, and mature stages), leek (*Allium porrum*), chive (*Allium schoenoprasum*), and ramson (*Allium ursinum*; selected plant parts) were comparatively evaluated in vitro. Lyophilized plant material was extracted with 50% ethanol/water. Antioxidant properties were determined by total phenolic content, DPPH radical scavenging activity, and FRAP reducing power. Antimicrobial activity was screened by agar well diffusion and quantified by broth dilution assays for bacteria and yeasts, while filamentous fungi were evaluated by a disc-based growth inhibition assay. Among the tested samples, ramson bulb exhibited the highest phenolic content (82.29 mg GAE/100 g fresh weight) and the strongest reducing power (FRAP-AS 603.78 μ M and FRAP-Fe 670.87 μ M per 100 g fresh weight), whereas the highest DPPH scavenging activity was recorded for ramson leaf (78.40%). In antibacterial assays, the lowest MIC/MBC against *Escherichia coli* (2.5/2.5 mg/mL) was observed for chive and ramson inflorescence extracts. Detectable activity against *Serratia marcescens* and *Micrococcus luteus* was restricted to ramson organs, with the inflorescence extract showing the highest potency (MIC/MBC 1.25/2.5 mg/mL). Overall, ramson plant parts and chive consistently showed the most pronounced bioactivities across the applied assays, indicating their relevance as candidates for further phytochemical characterization and application-oriented evaluation as natural sources of antioxidant and antimicrobial agents.

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Introduction

The genus *Allium* (Amaryllidaceae) comprises numerous species, several of which—such as onion (*Allium cepa*), leek (*A. porrum*), and chive (*A. schoenoprasum*)—have been widely consumed for centuries, while ramson or wild garlic (*A. ursinum*) has recently received increasing attention as a seasonal functional food. The phytochemical profile of *Allium* plants is dominated by organosulfur compounds and a broad phenolic fraction, including flavonoids (Corzo-Martínez et al. 2007). These constituents are considered major contributors to the reported antioxidant and antimicrobial properties of *Allium*-derived foods and preparations (Gorrepalli et al. 2024).

Organosulfur compounds are responsible for the characteristic taste and odor of onion and garlic. The main sulfur-containing constituents of *Allium* species are S-alk(en)yl-L-cysteine sulfoxides (ACSOs). In some compounds, such as alliin, the hydrocarbon moiety bound to sulfur contains a double bond, whereas in others, such as methiin, it is saturated. ACSOs are present in the cytosol

and may act as efficient scavengers, thereby contributing to secondary antioxidant effects. For example, alliin has been reported to trap acrolein, a cytotoxic reactive carbonyl compound, more effectively than epigallocatechin gallate (Hada et al. 2026).

Upon physical disruption of plant tissues, the enzyme alliinase comes into contact with ACSOs and converts them into the corresponding sulfenic acids. These intermediates are subsequently transformed into thiosulfinates, compounds with strong antimicrobial activity, and finally into dialkyl disulfides, such as diallyl disulfide and methyl allyl disulfide, which also contribute to antimicrobial activity and to the characteristic odor of *Allium* plants. Thiosulfinates can covalently block -SH moieties in the active centers of microbial enzymes, thereby interfering with lipid and RNA synthesis. Allicin, in particular, is effective against both Gram-positive and Gram-negative bacteria, although chain length may influence the relative activity against different microbial groups (Kyung and Lee 2001).

Phenolic compounds are also abundant in *Allium* species. Ferulic acid, p-hydroxybenzoic acid, p-coumaric

acid, and several kaempferol glycosides have been detected in ramson (Sobolewska et al. 2015). Other authors, such as Hedges and Lister (2007), described quercetin glycosides, anthocyanins, and flavanols in frequently consumed *Allium* species.

Natural antioxidants have attracted considerable interest in the fields of health preservation, disease prevention, anti-aging, and food protection. However, several factors complicate the establishment of a clear overall picture of the antioxidant and antimicrobial properties of *Allium* species.

Bioactivity within the genus *Allium* is strongly influenced by species, plant organ, origin, and extraction conditions. For *A. ursinum*, chemical and functional variability across organs and collection sites, together with measurable differences in phenolic and sulfur-related constituents, has been documented (Radušienė et al. 2025; Kovačević et al. 2023). Antibacterial effects likewise vary among samples and may be associated with sulfur chemistry. Burton et al. (2023), for example, found in a broad sampling set that allicin was the only metabolite positively associated with antibacterial activity. Quantitative susceptibility profiles of food-associated bacteria to *A. ursinum* extracts further highlight strain- and concentration-dependent effects (Stupar et al. 2022). Stage-dependent antibacterial activity of hydroethanolic ramson leaf extracts prepared with ethanol:water (30:70, v/v) has also been reported (Krisch et al. 2025), underscoring the importance of plant developmental stage and extraction parameters.

Onion (*A. cepa*) is consumed at different developmental stages. Young or spring onions are mild in taste, and their bulbs are only slightly developed; therefore, the green leaves and stems are consumed together. Summer onions have lost most of their leaves and possess developed bulbs but lack the dry outer scales typical of mature onions. Mature onions are ready for harvest and winter storage and are characterized by dry red outer scales.

For onion, recent literature emphasizes that different tissues and processing streams, including by-products such as peel or skin, represent distinct phytochemical reservoirs often enriched in flavonoids and phenolic acids with potential antioxidant and antimicrobial relevance (Stoica et al. 2023). Extraction solvent selection can substantially influence both chemical composition and microbiological activity in onion matrices (Joković et al. 2024), supporting the need for standardized hydroethanolic extraction protocols in comparative studies.

Antioxidant capacity is increasingly regarded as an assay-dependent composite trait shaped by multiple classes of compounds. In chive, tissue-resolved analyses have demonstrated pronounced differences in phenolic metabolism and antioxidant performance between green

and white tissues, indicating strong organ and tissue specificity even within a single *Allium* species (Dai et al. 2024). Moreover, detailed postharvest analyses indicate that different assays may emphasize different biochemical drivers: ascorbic acid may dominate DPPH scavenging and FRAP responses in some matrices, whereas phenolic acids may contribute more strongly to other radical-scavenging endpoints; organosulfur-related fractions may exhibit comparatively weak DPPH activity and limited FRAP capacity (Dai et al. 2023). These findings provide a clear rationale for applying multiple complementary assays, such as total phenolics, DPPH, and FRAP, when comparing *Allium*-derived materials.

Processing may also modulate bioactivity. For example, fermented onion and chive preparations have been reported to inhibit selected pathogenic bacteria with MIC/MBC values in the low mg/mL range for some strains (Hai et al. 2025).

Accordingly, the present study provides an integrated in vitro comparison of hydroethanolic extracts prepared from onion (*A. cepa*; young/spring, summer, and mature stages), leek (*A. porrum*), chive (*A. schoenoprasum*), and ramson (*A. ursinum*; selected organs). Antioxidant properties were assessed by total phenolic content (Folin-Ciocalteu), DPPH radical scavenging activity, and FRAP reducing power, whereas antimicrobial activity was evaluated by diffusion screening followed by MIC/MBC determination for bacteria and yeasts and by a disc-based growth inhibition assay for filamentous fungi. The aim was to establish a multi-endpoint activity profile that may support the evidence-based selection of *Allium* matrices for subsequent targeted phytochemical and application-oriented studies.

Materials and methods

Plant material and sample preparation

Plant materials representing commonly consumed *Allium* vegetables and herbs were used: onion (*Allium cepa* L.; young/spring onion, summer onion, and mature onion), leek (*A. porrum* L.), chive (*A. schoenoprasum* L.), and ramson (*A. ursinum* L.). In the ramson samples, plant parts were separated prior to processing (bulb, leaf, and inflorescence).

Plant materials were stored frozen at -20°C until processing and were then cut into small pieces, depending on sample type, either by grating or by knife cutting. To ensure complete freezing after cutting, samples were re-frozen for 24 h prior to freeze-drying.

Freeze-drying (lyophilization)

Frozen samples were freeze-dried using a laboratory

lyophilizer (LYOVAC GT 2, Seib Industry GmbH, Sweden) under the following conditions: storage temperature, -20°C ; freezing temperature, -40°C ; and evaporation temperatures of 35°C (heating) and -40°C (cooling). Dry matter yields ranged approximately from 8% to 34%, depending on sample type and plant part.

Preparation of hydroethanolic extracts

Extracts were prepared from the freeze-dried material under standardized conditions. Briefly, 2 g of dry matter was mixed with 40 mL of 50% (v/v) ethanol/water, vigorously shaken, and incubated for 24 h in the dark at room temperature. After incubation, the suspensions were vortex-homogenized and filtered through a paper filter (Schleicher & Schuell 597).

Sterility of the extracts, required for microbiological testing, was achieved by membrane filtration using syringe filters (Pall Acrodisc PF, 32 mm; $0.8/0.2\ \mu\text{m}$ membrane) into sterile, tightly closed tubes. The extracts were stored frozen at -20°C until analysis.

To determine total soluble solids in the extracts, 1 mL aliquots were dried to constant weight overnight at 60°C , and concentrations were calculated as mg dry extract per mL.

Microorganisms, media, and culture conditions

The test panel comprised Gram-negative bacteria (*Escherichia coli* and *Serratia marcescens*), Gram-positive bacteria (*Micrococcus luteus* and *Bacillus subtilis*), yeasts (*Saccharomyces cerevisiae* and *Pichia anomala*), and filamentous fungi (*Aspergillus niger* and *Fusarium* sp.). Strains were obtained from the Szeged Microbial Collection (SZMC, University of Szeged, Szeged, Hungary).

Bacteria were grown in LB or TGE broth, while yeasts and fungi were grown in malt broth. Solid media were prepared by supplementing the corresponding broths with agar. Media compositions per liter were as follows: TGE broth, glucose 10 g, peptone 5 g, yeast extract 2.5 g; TGE agar, TGE broth supplemented with 15 g agar. LB broth, casein peptone 10 g, NaCl 10 g, yeast extract 5 g; LB agar, LB broth supplemented with 12 g agar. Malt broth, glucose 20 g, malt extract 20 g, peptone 10 g; malt agar, malt broth supplemented with 15 g agar.

Overnight cultures were prepared as follows: *E. coli* in 50 mL LB; *S. marcescens* and *M. luteus* in 50 mL TGE at 37°C ; *B. subtilis* in TGE; and yeasts in malt broth at $28-30^{\circ}\text{C}$. Cell numbers were determined using a Bürker counting chamber and adjusted by dilution to approximately 10^5 cells/mL before agar inoculation and MIC testing.

Agar well diffusion assay

Agar plates appropriate for each test organism were poured under sterile conditions and allowed to dry for

24 h. The plates were surface-inoculated with 100 μL of standardized bacterial or yeast suspension (approximately 10^5 cells/mL) and allowed to absorb. Four wells were punched using a sterile 7 mm cork borer, and 100 μL of extract was added into three of the wells. The fourth well served as solvent control and contained the ethanol/water solution used for extraction. The plates were left to stand for 1 h to allow absorption and diffusion and were then incubated for 24 h at the appropriate temperature. Inhibition zones were measured in mm. Each condition was tested in triplicate and reported as mean $\pm$ SD.

For filamentous fungi, cultures were incubated on agar for 72 h to promote sporulation. Spores were washed off, suspended in 5 mL sterilized tap water, and used to inoculate malt agar plates. These plates were incubated at $28-30^{\circ}\text{C}$ and used for the diffusion assay as described above.

MIC/MBC determination for bacteria and yeasts

MIC testing was performed only for organisms that exhibited inhibition zones in the diffusion assay. Twofold dilution series, comprising five dilution steps, were prepared from stock extracts using sterilized tap water.

Bacteria and yeasts were grown for 24 h in broth and adjusted to approximately 10^5 cells/mL. For each dilution, 0.5 mL inoculum was mixed with 0.5 mL extract dilution (final volume 1.0 mL) in sterile closable tubes; three parallel tubes were prepared for each concentration. Following 24 h incubation, tubes were inspected visually, and the lowest concentration showing no visible growth (no turbidity) was recorded as the MIC.

To determine whether the MIC represented a static or killing endpoint, 100 μL aliquots from the MIC tube and from all higher concentrations were plated onto the appropriate agar and incubated for 24 h. The presence of growth on agar indicated a static MIC, whereas the absence of growth indicated a killing endpoint. In cases with no growth on agar, MIC equaled MBC (minimum bactericidal concentration) or MFC (minimum fungicidal concentration, for yeasts).

Growth inhibition assay for filamentous fungi

For filamentous fungi, growth inhibition was assessed using a paper disc method. Sterile filter paper discs (1 cm diameter) were loaded with 10 μL of each extract dilution, dried under sterile conditions, and placed onto plates inoculated with fungal spore suspension (three discs per dilution). The lowest concentration that produced a visible inhibition zone was recorded as the growth-inhibitory concentration in this assay format.

Preparation of extracts for antioxidant assays and replication scheme

For antioxidant assays, each sample was extracted in two parallel preparations (A and B), and each extract was measured in triplicate, giving a total of six measurements per sample. Based on preliminary spectrophotometric trials, stock extracts were diluted 1:1 by mixing 20 mL of concentrated extract with 20 mL of 50% ethanol to obtain working solutions (final volume 40 mL) used for Folin-Ciocalteu, DPPH, and FRAP assays.

Total phenolic content assay

Total phenolic content was determined by the Folin-Ciocalteu method. Reagents were prepared as follows: Na_2CO_3 (5 g/100 mL), gallic acid stock solution (1 mg/mL in 100% ethanol), and 50% Folin-Ciocalteu reagent prepared by 1:1 dilution with water. Calibration standards (0–100 $\mu\text{g/mL}$) were prepared from gallic acid in 100% ethanol. Absorbance was measured at 725 nm.

Reaction mixtures consisted of 200 μL diluted extract, 200 μL 100% ethanol, 1000 μL distilled water, and 500 μL 50% Folin-Ciocalteu reagent; after 5 min, 200 μL 5% Na_2CO_3 was added. Samples were incubated for 60 min at room temperature in the dark and measured at 725 nm. Results were expressed as mg gallic acid equivalents (GAE) per 100 g fresh weight (FW), based on recorded sample yields during lyophilization.

DPPH radical scavenging activity assay

A fresh 100 μM DPPH working solution was prepared prior to measurement by dissolving 39.5 mg DPPH in 10 mL of 96% ethanol to obtain a 10 mM stock solution, followed by dilution of 1 mL stock with 99 mL 96% ethanol. Absorbance was measured at 517 nm.

Reaction mixtures contained 200 μL diluted extract and 1200 μL 100 μM DPPH solution; the control contained 200 μL 96% ethanol instead of extract. After 30 min incubation at room temperature in the dark, absorbance was recorded at 517 nm. Radical scavenging activity was calculated as inhibition (%) = $[(A_{\text{control}} - A_{\text{sample}}) / A_{\text{control}}] \times 100$.

FRAP reducing power assay

FRAP (ferric reducing antioxidant power) was measured at 593 nm using a spectrophotometer thermostated to 37 °C. Reagents were prepared as follows: 300 mM acetate buffer (pH 3.6, stored at 4 °C), 40 mM HCl, 10 mM TPTZ (2,4,6-tris(2'-pyridyl)-1,3,5-triazine) in 40 mM HCl, and 20 mM $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$. The FRAP working reagent consisted of 40 mL acetate buffer, 4 mL TPTZ solution, 4 mL FeCl_3 solution, and 2.4 mL distilled water, and was kept at 37 °C during measurements.

For measurement, 30 μL diluted extract (or standard/

blank) was mixed with 1000 μL FRAP reagent and incubated for 30 min at 37 °C. Absorbance was then recorded at 593 nm. Calibration series were prepared from 1 mM ascorbic acid and 1 mM FeSO_4 standards. Results were expressed as μM ascorbic acid equivalents/100 g FW and μM Fe(II) equivalents/100 g FW.

Data handling

Agar diffusion data were obtained from three replicate measurements per condition and are reported as mean $\pm$ SD. MIC/MBC/MFC determinations were performed in triplicate tubes or discs per concentration, with endpoint definitions as described above. Antioxidant assays were based on two independently prepared extracts (A and B), each measured in triplicate, and are reported as mean $\pm$ SD.

Statistical analysis

Antioxidant assay data were analyzed by one-way ANOVA followed by post hoc pairwise comparisons in Excel 2016. Differences were considered significant at $p \leq 0.05$.

Results

Antioxidant activity

Marked differences were observed among the tested *Allium* extracts in all antioxidant assays (Table 1). Total phenolic content (TPC) covered a broad range, with the lowest value recorded for young/spring onion (12.80 mg GAE/100 g FW). Ramson bulb and ramson inflorescence showed the highest TPC values among the tested extracts. In agreement with the TPC pattern, reducing power assessed by FRAP was strongest for ramson bulb, followed by ramson inflorescence and chive. DPPH radical scavenging activity showed a partly similar ranking: the highest scavenging activity was measured for ramson leaf (78.40%), whereas young/spring onion again displayed the lowest value (2.14%). Among the onion samples, summer onion showed higher FRAP values than mature and young/spring onion, whereas mature onion showed higher DPPH scavenging activity than summer onion. Overall, ramson organs and chive represented the most active group across antioxidant assays, while the onion samples, particularly young/spring onion, showed comparatively lower antioxidant performance.

Antimicrobial activity

Quantitative antimicrobial activity varied strongly according to both microorganism and extract. For bacteria and yeasts (Table 2), the lowest MIC/MBC values against *E. coli* were obtained for chive and ramson inflorescence extracts (2.5/2.5 mg/mL), whereas onion samples and leek

Table 1. Antioxidant capacity of hydroethanolic extracts from selected *Allium* samples. Values are mean $\pm$ SD (n = 6).

Sample	TPC (mg GAE/100 g FW)	DPPH scavenging (%)	FRAP-AS (μ M AS/100 g FW)	FRAP-Fe (μ M Fe(II)/100 g FW)
Spring onion (<i>A. cepa</i> , young)	12.80 $\pm$ 1.58 f	2.14 $\pm$ 0.74 e	100.70 $\pm$ 2.19 g	125.79 $\pm$ 1.84 g
Summer onion (<i>A. cepa</i>)	28.47 $\pm$ 5.42 b,c,d	9.92 $\pm$ 2.13 a	253.28 $\pm$ 16.04 e	298.31 $\pm$ 5.51 e
Mature onion (<i>A. cepa</i> , red)	19.89 $\pm$ 4.83 d	18.34 $\pm$ 2.04 f	165.07 $\pm$ 9.48 f	193.70 $\pm$ 9.18 f
Leek (<i>A. porrum</i>)	35.27 $\pm$ 1.58 c	4.18 $\pm$ 2.13 e	154.39 $\pm$ 5.83 f	179.01 $\pm$ 5.51 f
Chive (<i>A. schoenoprasum</i>)	60.31 $\pm$ 4.44 e	28.24 $\pm$ 2.59 d	457.01 $\pm$ 16.04 b	506.61 $\pm$ 15.60 b
Ramson leaf (<i>A. ursinum</i>)	29.45 $\pm$ 2.76 b	78.40 $\pm$ 1.67 b	331.01 $\pm$ 8.75 d	373.55 $\pm$ 8.26 d
Ramson inflorescence (<i>A. ursinum</i>)	80.12 $\pm$ 4.83 a	14.36 $\pm$ 2.41 a	415.36 $\pm$ 16.77 c	468.07 $\pm$ 15.60 c
Ramson bulb (<i>A. ursinum</i>)	82.29 $\pm$ 7.79 a	52.03 $\pm$ 2.04 c	603.78 $\pm$ 17.50 a	670.87 $\pm$ 15.60 a

TPC: total phenolic content; DPPH: radical scavenging activity; FRAP-AS: ferric reducing power expressed as μ M ascorbic acid equivalents/100 g FW; FRAP-Fe: ferric reducing power expressed as μ M Fe(II) equivalents/100 g FW. GAE gallic acid equivalents; FW: fresh weight. Different letters represent significant differences in a column ($p \leq 0.05$).

required higher concentrations for growth inhibition and/or killing. Activity against *S. marcescens* was detected exclusively in ramson organs, with ramson inflorescence showing the highest potency (MIC/MBC 1.25/2.5 mg/mL). In the case of the Gram-positive *M. luteus*, inhibition was observed for onion samples and ramson organs, again with the strongest effect for ramson inflorescence (MIC/MBC 2.5/2.5 mg/mL). In yeast assays, inhibition of *P. anomala* was most pronounced for ramson inflorescence (MIC/MFC 1.25/2.5 mg/mL) and chive (5/5 mg/mL), whereas the other extracts showed weaker activity or no detectable activity within the tested concentration range. Under the applied conditions, no inhibitory effects were detected for *B. subtilis* or *S. cerevisiae*.

In the disc-based assay for filamentous fungi (Table 3), measurable growth inhibition against *A. niger* was observed for chive, ramson bulb, ramson inflorescence, and ramson leaf. Chive showed the strongest activity, with visible inhibition at 5 mg/mL, followed by ramson inflorescence at 10 mg/mL, ramson leaf at 20 mg/mL, and ramson bulb at 50 mg/mL. No inhibitory activity was detected for

Fusarium sp. Overall, the antimicrobial dataset identified *E. coli* as the most broadly susceptible bacterium across the tested extracts, whereas *S. marcescens* inhibition was restricted to ramson organs; antifungal activity was more selective and was detectable only against *A. niger* in certain matrices.

Discussion

The results demonstrated pronounced species- and organ-dependent differences in both antioxidant capacity and antimicrobial activity among the tested hydroethanolic *Allium* extracts. Ramson plant parts and chive consistently ranked among the strongest samples across multiple antioxidant assays, whereas onion samples, particularly young/spring onion, showed lower antioxidant performance. This pattern agrees with the broader view that *Allium* bioactivity depends strongly on plant matrix and extraction conditions, and that methodological standardization is essential for reproducible, application-

Table 2. Minimum inhibitory (MIC) and minimum bactericidal/fungicidal concentrations (MBC/MFC) of hydroethanolic extracts from selected *Allium* samples against bacteria and yeasts. Values are given in mg/mL.

Extract	<i>E. coli</i> MIC	<i>E. coli</i> MBC	<i>S. marcescens</i> MIC	<i>S. marcescens</i> MBC	<i>M. luteus</i> MIC	<i>M. luteus</i> MBC	<i>P. anomala</i> MIC	<i>P. anomala</i> MFC
Spring onion	5	10	–	–	–	–	–	–
Summer onion	3.75	7.50	–	–	7.50	15	–	–
Mature onion	7.50	7.50	–	–	15	15	–	–
Leek	5	5	–	–	–	–	–	–
Chive	2.50	2.50	–	–	–	–	5	5
Ramson bulb	6.25	12.50	6.25	12.5	12.50	25	12.50	12.50
Ramson inflorescence	2.50	2.50	1.25	2.50	2.50	2.50	1.25	2.50
Ramson leaf	5	10	5	5	5	5	5	5

No inhibition at the highest concentration tested is marked with –.

Table 3. Lowest extract concentration producing a visible inhibition zone against *Aspergillus niger* in the disc-based assay. Values are given in mg/mL.

Extract	Lowest concentration with visible inhibition zone	Fungicidal endpoint
Spring onion	–	ND
Summer onion	–	ND
Mature onion	–	ND
Leek	–	ND
Chive	5	ND
Ramson bulb	50	ND
Ramson inflorescence	10	ND
Ramson leaf	20	ND

No inhibition at the highest concentration tested is marked with –
ND: not determined.

oriented evaluation (Gorrepati et al. 2024).

Antioxidant rankings differed among assays: ramson bulb showed the highest total phenolic content and the strongest reducing power in the FRAP assay, whereas the highest DPPH scavenging activity was observed for ramson leaf. Such divergence is biologically plausible and consistent with mechanistic evidence from chive indicating that DPPH and FRAP responses may be driven to different extents by ascorbic acid and specific phenolic acids, while organosulfur-related fractions may contribute less to these endpoints (Dai et al. 2023). Tissue-part comparisons further support the view that antioxidant performance can be strongly tissue-dependent within *Allium* species (Dai et al. 2024). From a methodological perspective, the observed differences among TPC, DPPH, and FRAP values are also expected because the Folin-Ciocalteu assay reflects general reducing capacity rather than phenolics exclusively (Singleton et al. 1999), whereas DPPH and FRAP probe different reaction mechanisms (Brand-Williams et al. 1995; Benzie and Strain 1996). Therefore, the partial divergence among these endpoints should be regarded as complementary rather than contradictory information.

The strong antioxidant performance of ramson organs is consistent with evidence that the chemistry of *A. ursinum* varies with plant organ and habitat, thereby influencing measured antioxidant outcomes (Radušienė et al. 2025; Kovačević et al. 2023). Cross-study differences have also been attributed to extraction solvent and sample origin, emphasizing the matrix- and method-dependent nature of wild garlic preparations (Szczepaniak et al. 2025). For onion-derived materials, reviews have highlighted that peel and skin fractions can be enriched in flavonoids and phenolic acids with potential functional relevance (Stoica et al. 2023), and that solvent selection can markedly influence recovered composition and biological activity (Joković et al. 2024). Together, these

data support the use of a fixed hydroethanolic extraction protocol for within-study comparison, while also underlining that extraction parameters remain major determinants of the observed bioactivity.

In antimicrobial assays, the strongest quantitative effects were observed against *E. coli*, with the lowest MIC/MBC values measured for chive and ramson inflorescence extracts (2.5/2.5 mg/mL; Table 2). MIC values in the mg/mL range are typical for crude plant extracts; for example, *A. ursinum* extracts prepared by alternative extraction approaches have shown MIC/MBC values frequently in the tens of mg/mL range against food-associated bacteria, with clear strain- and concentration-dependent effects (Stupar et al. 2022). In this context, the comparatively low MIC values observed here for selected matrices are consistent with organ-dependent enrichment of inhibitory constituents. In addition, stage-dependent antibacterial activity of hydroethanolic ramson leaf extracts prepared with ethanol:water (30:70, v/v) has been reported previously (Krisch et al. 2025), supporting the relevance of developmental stage and extraction conditions when interpreting between-study differences.

A particularly notable pattern was that inhibition of *S. marcescens* and *M. luteus* was restricted to ramson organs, with the strongest effect observed for the inflorescence extract. Such organism-specific selectivity is common in crude plant extracts. Recent work on *A. ursinum* further suggests that antibacterial variability can be chemically anchored: across a broad sampling set, allicin was the only metabolite positively associated with antibacterial activity (Burton et al. 2023). Reviews also emphasize the importance of thiopolysulfides and related sulfur chemistry and highlight the strong dependence of antimicrobial activity on origin and extraction conditions (Szczepaniak et al. 2025). These observations provide a plausible mechanistic context for the organ-dependent antimicrobial patterns observed here and support follow-up studies combining activity testing with targeted profiling of sulfur compounds and phenolics.

Interpretation of antimicrobial results should also take methodological differences between diffusion screening and broth dilution endpoints into account. Agar diffusion depends not only on the potency of active compounds but also on their diffusibility and stability in the agar matrix; therefore, inhibition zones are not necessarily directly rank-consistent with MIC/MBC values (Balouiri et al. 2016). This is particularly relevant for *Allium* matrices, in which reactive sulfur compounds may be unstable and assay-format dependent.

Finally, selected extracts also showed measurable activity against *P. anomala* and *A. niger*, whereas *Fusarium* sp. showed no detectable inhibition under the applied conditions. This profile is consistent with current mech-

anistic syntheses indicating that antifungal responses to *Allium* constituents vary widely according to fungal species, strain, and compound class (Eslami et al. 2025). Overall, the present work establishes a harmonized multi-endpoint activity profile for selected *Allium* matrices under a fixed hydroethanolic extraction protocol and identifies ramson organs and chive as high-priority candidates for subsequent targeted phytochemical characterization and application-oriented testing.

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