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Original Article

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Antibacterial Effect of *Allium ampeloprasum* and *Allium porrum* Extracts on *Staphylococcus aureus* and *Pseudomonas aeruginosa*

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Objectives: The most prominent microorganisms that cause hospital infections and acquire antibiotic resistance are *Staphylococcus aureus* and *Pseudomonas aeruginosa*. The present study aimed to compare the phenolic and flavonoid compounds of various *Allium ampeloprasum* and *Allium porrum* extracts and evaluate the antibacterial effects of these extracts against these two microorganisms.
Methods: The total phenolic and flavonoid contents of the acetone, methanol, aqueous, and hexane leeks extracts from *A. ampeloprasum* and *A. porrum* were measured. The antibacterial activity of these extracts against *S. aureus* and *P. aeruginosa* was tested using the disk diffusion method for 24, 48, and 72 hours. Further, the minimum inhibitory concentrations and the minimum bactericidal concentrations of these extracts for these two bacteria were evaluated and compared with those of common antibiotics.
Results: The aqueous extracts showed the highest phenolic and flavonoid contents and at concentrations of 35 and 40 mg per disk, showed the most antibacterial activity against *S. aureus* and *P. aeruginosa*; *P. aeruginosa* showed more sensitivity to the aqueous extracts than *S. aureus*.
Conclusion: Aqueous *A. ampeloprasum* and *A. porrum* extracts may prevent the growth of hospital pathogens, especially *P. aeruginosa*; our findings will aid the discovery of new antimicrobial substances against antibiotic-resistant bacteria.

Keywords: antibiotic resistance, leek, phytotherapy, *pseudomonas aeruginosa*, *staphylococcus aureus*, traditional persian medicine

INTRODUCTION

Hospitals and healthcare settings represent one of the most important reservoirs for the transmission of pathogenic bacteria [1]. The most common cause of hospital infections in patients is *Pseudomonas aeruginosa*, which is present in medical devices, such as anesthesia and dialysis equipment, inhalers, oxygen respirators, vaporizers, and humidifiers [2, 3]. It is a gram-negative pathogenic bacterium belonging to the *Pseudomonadaceae* family and is rod-shaped and non-spore-forming, with a single polar flagellum [2]. *Pseudomonas aeruginosa* can cause a wide variety of life-threatening acute and chronic infections, including cystic fibrosis, urinary tract infections, ventilator-associated pneumonia, bone and joint infections, otitis externa, bacteremia, systemic infections, burns, and wound injuries [4-8].

Another one of the primary causes of infections related to healthcare is *Staphylococcus aureus* [9]. It is a gram-positive bacterium belonging to the *Pseudomonadaceae* family and is

non-spore-forming, spherical, and non-motile, appearing as grape-like clusters [10, 11]. This pathogen is a significant contributor to bacteremia, infective endocarditis, pleural, bone, skin, and soft tissue infections, as well as infections related to medical devices [9].

Most hospital infections are caused by antibiotic-resistant bacteria that have developed from the usage of chemical antibiotics [12]. Medicinal plants play a valuable role in promoting community health for the treatment and prevention of disease [13, 14]. In recent years, the use of medicinal plants as natural sources of antibacterial substances has received an increasing amount of attention. In addition to limiting the development of antibiotic-resistant bacteria, using such antibacterial sources can eliminate side effects caused by the use of chemical antibiotics [15]. Wild leek (*Allium ampeloprasum* var. porrum) and elephant garlic (*Allium ampeloprasum* var. ampeloprasum) belong to the Allium family, a large genus of onion- or garlic-scented bulbous plants of the *Amaryllidaceae* family [16]. Several studies have shown that these plants have many medicinal properties, including antioxidant and antimicrobial effects [17-21].

The present study aims to evaluate the antibacterial effects of various extracts of wild leek and elephant garlic and compare them with those of antibiotics commonly used against *P. aeruginosa* and *S. aureus*.

MATERIALS AND METHODS

1. Plant collection and extraction

Allium ampeloprasum and *A. porrum* were purchased from a greenhouse in Chadegan county, Isfahan province, Iran, and transferred to the Institute of Traditional Medicine and Herbal Plants in Esfahan, Iran. The plants were washed twice, and the roots and stalks were chopped and dried for eight days in the shade at the optimum lab temperature. The dry components were ground into a powder in a laboratory grinder and passed through a mesh (80 and 100 sizes). The percolation method was used to prepare the different extracts. First, the plant powders (50 g) were added to different solvents: methanol, acetone, water, and hexane, and soaked for 72 hours. Then, using Whatman filter paper (pore size, 11 μm), the liquid components of each extract were separated, evaporated, and stored at 4℃ until further use.

2. Determination of total phenolic contents

The total phenolic contents of the different *A. ampeloprasum* and *A. porrum* extracts were measured using the Folin–Ciocalteu method. The absorbance of the extracts was determined at 700 nm; gallic acid (Sigma Aldrich) was used as the standard. The total phenolic content was presented as the gallic acid equivalent (GAE) per gram of the dry weight of the extract (GAE/g).

3. Determination of total flavonoid contents

The total flavonoid contents of the different *A. ampeloprasum* and *A. porrum* extracts were measured by the aluminum chloride assay. The absorbance of the extracts was measured at 405 nm. The total flavonoid content was determined based on a standard calibration method using quercetin as the standard; the results were expressed as the quercetin equivalent (QE) per gram of dry weight of the extract (QE/g).

4. Antimicrobial activity assays

1) Microorganisms

Cultures of *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (PTCC 1310) were prepared at the Alternative Medicine Institute of Isfahan, Iran. Muller–Hinton agar (MHA) was used as the standardized solid medium; MHA was transferred to 5-cm-thick sterile Petri dishes. Then, the bacterial samples were inoculated in the agar plates medium under sterile conditions.

2) Antibacterial assay

Among the various extracts prepared using different solvents, the aqueous extracts, which showed the highest total phenolic and flavonoid contents, were chosen for further antimicrobial testing to ascertain the antibacterial activity of *A. ampeloprasum* and *A. porrum*. The disk diffusion technique, performed via the Kirby–Bauer method, was employed to measure the antibacterial activity. The minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of the aqueous *A. ampeloprasum* and *A. porrum* extracts were also determined. The bacteria were cultured in MHA and the density of the suspension (in MHA) was adjusted to 0.5 McFarland, followed by incubation for 18 hours at 37℃ to assess the diameter of the zones of inhibition. Then, 500 μL

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of the bacterial suspension was gently spread onto the surface of MHA plates using a sterile loop. Various concentrations of the aqueous *A. ampeloprasum* and *A. porrum* extracts (2.5, 5, 10, 15, 20, 25, 30, 35, and 40 mg/disk) were added to the plates. The positive control groups received tetracycline (30 μg/disk), ampicillin, and penicillin (10 μg/disk). The negative control group received no extracts or antibiotics. After incubation at 37℃ for 24, 48, and 72 hours, the inhibition zone diameters (mm) were determined. Three duplicates were maintained for each group, and microbial growth was assessed using a microtiter plate reader at 600 nm (Multiskan Ascent, Labsystems, Helsinki, Finland).

5. Statistical analysis

Data were analyzed using a one-way ANOVA by GraphPad Prism Version 6 software (GraphPad Software, La Jolla, CA, USA). Tukey's multiple comparison tests were used to compare the means. The differences were considered significant at $p < 0.05$.

RESULTS

The aqueous and the hexane extracts of *A. ampeloprasum* and *A. porrum* showed the highest and lowest phenolic and flavonoid contents, respectively ($p < 0.05$, Figs. 1, 2).

Data regarding the antibacterial activities of the aqueous

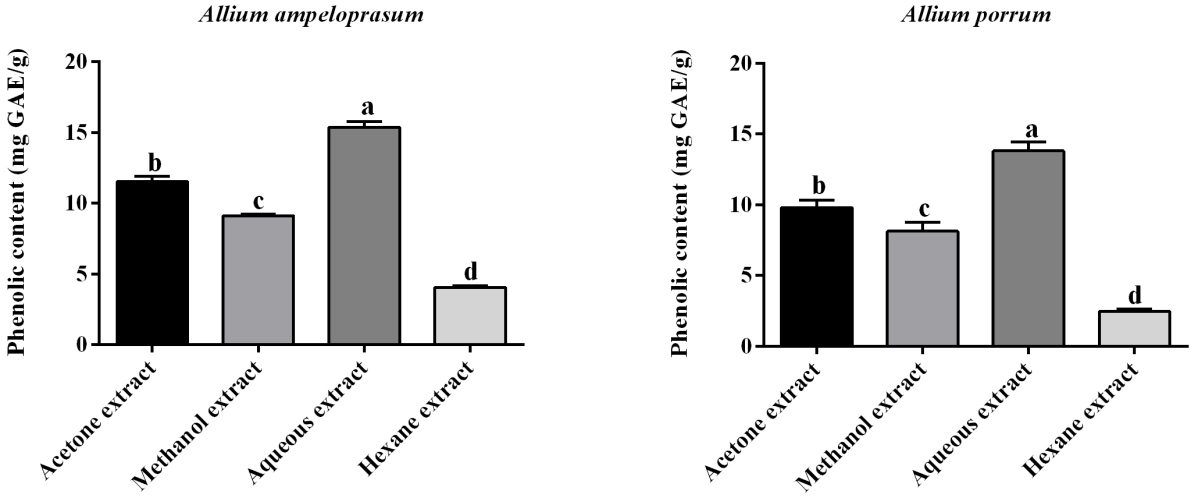

Figure 1. Total phenolic contents of *Allium ampeloprasum* and *Allium porrum* extracts.

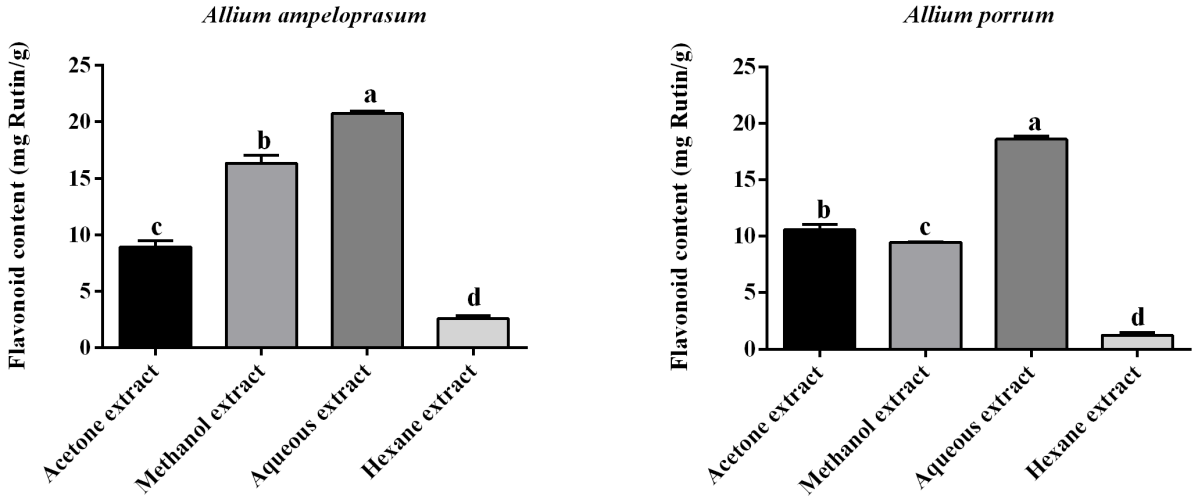

Figure 2. Total flavonoid contents of *Allium ampeloprasum* and *Allium porrum* extracts.

extract of *A. ampeloprasum* against *S. aureus* and *P. aeruginosa* showed that the minimum concentration showing antibacterial effects was 10 mg per disk (Table 1). The diameter of the zones of inhibition for the two bacteria increased with the increase in the extract concentration; the maximum inhibition was observed against *S. aureus* at concentrations of 35 and 40 mg per disk and against *P. aeruginosa* at a concentration of 40 mg per disk ($p < 0.05$, Table 1). Overall, there were no significant differences between the inhibition zone diameters after incubation for 24, 48, and 72 hours.

A. Purium aqueous extract exhibited minimum antibacterial activity against *S. aureus* and *P. aeruginosa* at concentrations of 15 and 10 mg per disk, respectively, according to our findings (Table 2). Furthermore, this extract had the most antibacterial effects against *S. aureus* and *P. aeruginosa* at concentrations of 35 and 40 mg per disk and 40 mg per disk, respectively ($p < 0.05$, Table 2). Antibacterial activities of different concentrations of aqueous extract of *A. perium* were similar at 24, 48, and 72 hours.

The MICs and MBCs of the aqueous extracts of *A. ampeloprasum* and *A. porrum* and the tested antibiotics against *S. aureus* and *P. aeruginosa* are shown in Table 3. *P. aeruginosa* showed more sensitivity to the *A. ampeloprasum* and *A. porrum* extracts than *S. aureus*.

Comparison of the antibacterial activity of the aqueous extracts of *A. ampeloprasum* and *A. porrum* at concentrations of 40 mg per disk with that of different antibiotics showed that tetracycline had the highest antibacterial activity against *S.*

Table 1. Antibacterial activity (mm) of *Allium ampeloprasum* aqueous extract against *Staphylococcus aureus* and *Pseudomonas aeruginosa*

Allium ampeloprasum (mg/disk)	*Staphylococcus aureus*			*Pseudomonas aeruginosa*		
	24 h	48 h	72 h	24 h	48 h	72 h
2.5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
10	0.67 ± 1.15^{a}	0.83 ± 1.44^{a}	0.83 ± 1.44^{a}	3.83 ± 0.21^{b}	4.17 ± 0.29^{b}	4.23 ± 0.25^{b}
15	4.23 ± 0.25^{b}	4.50 ± 0.30^{b}	4.73 ± 0.21^{b}	6.07 ± 0.40^{c}	6.80 ± 0.26^{c}	6.83 ± 0.28^{c}
20	6.30 ± 0.36^{c}	6.40 ± 0.53^{b}	6.40 ± 0.53^{b}	9.43 ± 0.51^{d}	10.17 ± 0.29^{d}	10.17 ± 0.29^{d}
25	10.13 ± 0.32^{d}	10.33 ± 0.58^{c}	10.50 ± 0.50^{c}	12.10 ± 0.56^{e}	12.53 ± 0.47^{e}	12.53 ± 0.47^{e}
30	11.00 ± 1.00^{d}	$12.17 \pm 0.76^{c,d}$	12.33 ± 1.04^{d}	13.90 ± 0.95^{f}	14.17 ± 0.76^{f}	14.33 ± 0.58^{f}
35	13.00 ± 1.00^{e}	$13.00 \pm 1.00^{d,e}$	$13.00 \pm 1.00^{d,e}$	15.67 ± 0.42^{g}	16.17 ± 0.29^{g}	16.17 ± 0.29^{g}
40	14.00 ± 0.50^{e}	14.57 ± 0.11^{e}	14.57 ± 0.11^{e}	17.30 ± 0.52^{h}	17.33 ± 0.58^{g}	17.33 ± 0.58^{h}

Results were presented as mean ± SD.

[a-h]In each column, different letters represent significantly difference at $p < 0.05$.

Table 2. Antibacterial activity (mm) of *A. porrum* aqueous extract against *S. aureus* and *P. aeruginosa*

Allium porrum (mg/disk)	*Staphylococcus aureus*			*Pseudomonas aeruginosa*		
	24 h	48 h	72 h	24 h	48 h	72 h
2.5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
5	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
10	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	2.10 ± 0.65^{b}	2.23 ± 0.75^{b}	2.23 ± 0.75^{b}
15	1.50 ± 1.80^{a}	2.00 ± 1.73^{a}	2.00 ± 1.73^{a}	4.23 ± 0.49^{c}	4.33 ± 0.58^{c}	4.33 ± 0.58^{c}
20	4.03 ± 0.45^{b}	4.33 ± 0.58^{b}	4.33 ± 0.58^{b}	7.33 ± 0.29^{d}	7.50 ± 0.30^{d}	7.50 ± 0.30^{d}
25	7.03 ± 0.25^{c}	7.33 ± 0.29^{c}	7.33 ± 0.29^{c}	8.90 ± 0.85^{e}	9.00 ± 1.00^{e}	9.00 ± 1.00^{e}
30	8.23 ± 0.25^{c}	8.90 ± 0.10^{c}	8.97 ± 0.06^{c}	10.93 ± 0.40^{f}	11.17 ± 0.29^{f}	11.17 ± 0.29^{f}
35	11.10 ± 0.98^{d}	11.70 ± 0.52^{d}	11.70 ± 0.52^{d}	13.10 ± 0.36^{g}	13.23 ± 0.25^{g}	13.23 ± 0.25^{g}
40	11.90 ± 0.66^{d}	12.10 ± 0.53^{d}	12.10 ± 0.53^{d}	14.60 ± 0.36^{h}	15.17 ± 0.29^{h}	15.17 ± 0.29^{h}

Results were presented as mean ± SD.

[a-h]In each column, different letters represent significantly difference at $p < 0.05$.

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Table 3. MIC and MBC values of *A. ampeloprasum*, *A. porrum*, and common antibiotics on *S. aureus* and *P. aeruginosa*

Component	MIC	MBC	Pathogen
A. ampeloprasum (mg/mL)	15	23	*Pseudomonas aeruginosa*
A. porrum (mg/mL)	20	31	
Ampicilin (μg/mL)	11	22	
Penicillin (μg/mL)	12	20	
Tetracycline (μg/mL)	6	11	
A. ampeloprasum (mg/mL)	24	40	*Staphylococcus aureus*
A. porrum (mg/mL)	29	46	
Ampicilin (μg/mL)	4	6	
Penicillin (μg/mL)	6	9	
Tetracycline (μg/mL)	5	7	

Table 4. Antibacterial activity (mm) of *A. ampeloprasum* and *A. porrum* aqueous extracts compared with antibiotics

Treatment (mg/disk)	*Staphylococcus aureus*			*Pseudomonas aeruginosa*		
	24 h	48 h	72 h	24 h	48 h	72 h
Allium ampeloprasum	14.00 ± 0.50^{b}	14.57 ± 0.11^{b}	14.57 ± 0.11^{b}	17.30 ± 0.52^{a}	17.33 ± 0.58^{a}	17.33 ± 0.58^{a}
Allium porrum	11.90 ± 0.66^{a}	12.10 ± 0.53^{a}	12.10 ± 0.53^{a}	14.60 ± 0.36^{b}	15.17 ± 0.29^{b}	15.17 ± 0.29^{b}
Ampicillin	15.90 ± 0.56^{b}	16.27 ± 0.64^{b}	16.50 ± 0.50^{b}	5.05 ± 0.48^{c}	5.57 ± 0.58^{c}	5.57 ± 0.58^{c}
Penicillin	13.70 ± 0.44^{a,b}	14.17 ± 0.76^{a,b}	14.33 ± 0.76^{a,b}	6.23 ± 0.25^{c}	6.40 ± 0.53^{c}	6.40 ± 0.53^{c}
Tetracycline	18.23 ± 1.25^{c}	18.50 ± 1.32^{c}	18.50 ± 1.32^{c}	5.70 ± 0.61^{c}	6.00 ± 0.50^{c}	6.00 ± 0.50^{c}

Results were presented as mean ± SD.
$^{a-c}$In each column, different letters represent significantly difference at $p < 0.05$.

aureus at different time intervals ($p < 0.05$, Table 4). Further, the results showed that the antibacterial activity of the *A. ampeloprasum* extract against *P. aeruginosa* was higher than that of the *A. porrum* extract ($p < 0.05$) after different time periods and was similar to the antibacterial activity of penicillin and ampicillin (Table 4). However, the antibacterial activity of the aqueous *A. ampeloprasum* and *A. porrum* extracts was higher than that of the antibiotics at different time intervals ($p < 0.05$). Moreover, the aqueous extract of *A. ampeloprasum* showed a higher degree of antibacterial activity against *P. aeruginosa* than the aqueous extract of *A. porrum* ($p < 0.05$). The antibiotics tetracycline, penicillin, and ampicillin displayed the same degree of antibacterial activity against *P. aeruginosa*.

DISCUSSION

The findings of this study revealed that the aqueous extracts of *A. ampeloprasum* and *A. porrum* showed antibacterial activity, particularly against *P. aeruginosa*. Our study also showed that the tested antibiotics (ampicillin, penicillin, and tetracycline) had a greater inhibitory effect against the growth of pathogens than the herbal extracts. Given the widespread therapeutic potential and low toxicity of herbal products, medicinal plants have attracted a great amount of interest recently. Furthermore, these natural resources are inexpensive and environment friendly. The antibacterial properties of onions and extracts from onion-like plants, such as leeks, have been proven [21-23]. All species of *Allium* contain thiosulfinate; *A. porrum* contains up to 0.15 micromol/g of thiosulfinate. This component has been demonstrated to exert antibacterial and antifungal activity against several gram-positive and -negative microorganisms [24]. Many organosulfur compounds, including glutamyl peptides, have been found in *A. ampeloprasum* [25]. In addition to saponins, the leaves and bulbs of *A. ampeloprasum* contain high amounts of polyphenols, phenolic acid, flavonoids, and tannins [26]. Similarly, a previous study revealed that trans-caryophyllene from members of the genus *Allium* showed antibacterial activities against *E. coli*, *P. aeruginosa*, *S. aureus*, and *Bacillus cereus in vitro* [27, 28]. Consistent with our findings, it has been previously shown that the aqueous extract

of elephant garlic prevented the growth of five bacteria: *Bacillus subtilis*, *Pseudomonas aeruginosa*, *Escherichia coli*, *Enterococcus faecalis*, and *Staphylococcus aureus* [15]. The present study demonstrated that the MICs of *A. ampeloprasum* and *A. porrum* extract were 24 and 29 mg/mL, respectively, against *S. aureus* and 15 and 20 mg/mL, respectively, against *P. aeruginosa*. Saeedi et al. (2017) [29] tested the effects of *A. ampeloprasum* extract against *Escherichia coli*, *S. aureus*, *Salmonella typhi*, *Bacillus cereus*, and *S. taphylococcus*, reporting MICs of 64, 32, 128, 64, and 16 mg/mL, respectively, against these organisms. Another research by Caputo et al. (2020) [30] demonstrated that *A. ampeloprasum* inhibited the growth of different pathogenic bacteria, displaying MICs of 18, 10, and 5 mg/mL against *Pectobacterium carotovorum*, *Listeria monocytogenes*, and *S. aureus*, respectively. These authors also showed that the methanol extract of *A. ampeloprasum* was highly effective in inhibiting the growth of three pathogenic bacteria (*Pectobacterium carotovorum*, *Listeria monocytogenes*, and *S. aureus*). Changes in the MIC values demonstrate a direct relationship with bacterial resistance, the amounts of compounds or active forms of flavonoids present in the extract, and the antimicrobial test method used [31].

CONCLUSION

Extracts from *A. ampeloprasum* and *A. allium* demonstrated antibacterial effects against *P. aeruginosa* and *S. aureus*; *P. aeruginosa* was more sensitive to these extracts than *S. aureus*. Our findings can lead to the discovery of new types of antimicrobial substances against antibiotic-resistant bacteria to prevent the spread of nosocomial pathogens.

CONFLICT OF INTEREST

All authors declare no conflict of interest.

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