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Short Communication

Antibacterial Activity of Ethanolic and Aqueous Extracts of *Echium amoenum* Against Human and Plant Pathogenic Bacteria

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Abstract

Echium amoenum is a traditional medicinal plant that has been used for its therapeutic properties, yet its antibacterial potential remains insufficiently studied. This research aimed to evaluate the antibacterial activity of ethanolic and aqueous extracts of *E. amoenum* against human pathogens (*Staphylococcus aureus, Salmonella* Typhi*, Escherichia coli*) and plant pathogens (*Brenneria nigrifluens, Erwinia amylovora, Serratia marcescens, Pseudomonas syringae*). Extracts were prepared by maceration and tested at concentrations of 50, 100, and 200 mg/mL using the agar well diffusion method. Both ethanolic and aqueous extracts exhibited dose-dependent inhibitory effects, with significantly larger inhibition zones at higher concentrations. The ethanolic extract showed stronger antibacterial activity compared to the aqueous extract. At 200 mg/mL, inhibition zones reached up to 21.6 mm for *S. marcescens* and 19.3 mm for *E. amylovora*, while *B. nigrifluens* and *E. coli* displayed the lowest susceptibility (7.6 mm and 12.6 mm, respectively). These findings indicate that *E. amoenum*, particularly its ethanolic extract, possesses considerable antibacterial potential against both human and plant pathogens. The results highlight its possible applications as a natural antimicrobial agent in medicine and agriculture, providing an alternative to synthetic antibiotics and chemical bactericides. Further *in vivo* and mechanistic studies are recommended to validate these effects and explore practical applications.

Keywords: *Echium amoenum*, antibacterial activity, aqueous extract, ethanolic extract, human pathogens, plant pathogens.

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Introduction

The rise of antibiotic-resistant bacteria is a major global health challenge. Overuse and misuse of antibiotics have led to strains of human pathogens that no longer respond to standard treatments, thereby increasing morbidity, mortality, and healthcare costs (1). In agriculture, bacterial pathogens likewise threaten crop yields and food security, particularly as plant resistance and effective chemical control become limited or environmentally undesirable (2). Consequently, there is growing interest in exploring natural sources of antimicrobial agents, including medicinal plants, which often contain diverse bioactive compounds such as phenolics, flavonoids, alkaloids, and essential oils. These compounds may offer novel mechanisms of action, reducing the likelihood of resistance development (3).

Echium amoenum (Boraginaceae), a perennial plant native to Iran, has been used traditionally for its multiple medicinal properties. Research has documented its antioxidant, anti-inflammatory, antiviral, and anticancer effects. For instance, its anthocyanin-rich extracts protect human endothelial cells from oxidative damage (4). Hexane, ethyl acetate, and dichloromethane extracts of *E. amoenum* flowers reduce inflammatory mediators in macrophages (e.g. IL-1β, IL-6, TNF-α, iNOS, COX-2) (5). In addition, there is evidence of antiviral activity and a significant antibacterial effect of aqueous and ethanolic extracts of *E. amoenum* against several Gram-positive and Gram-negative strains (6).

While many studies have addressed common human pathogenic bacteria, there is less information on plant pathogenic bacteria such as *Erwinia amylovora*, *Brenneria nigrifluens*, *Serratia marcescens*, and others. *E. amylovora* is the causal agent of fire blight in apple and pear (7), *B. nigrifluens* is responsible for bark canker in walnut (8), and *S. marcescens* and *Pseudomonas syringae* are opportunistic pathogens affecting a wide range of crops (9,10).

Among human pathogens, *Staphylococcus aureus* is a major cause of hospital- and community-acquired infections (11), *Salmonella* Typhi is the etiological agent of typhoid fever (12), and *Escherichia coli* includes strains associated with urinary tract and gastrointestinal infections (13). These pathogens cause economically and clinically important diseases, and their control is increasingly difficult under environmental and regulatory constraints (14).

In this context, the present study aims to compare the antibacterial effects of aqueous and ethanolic extracts of *Echium amoenum* against both human pathogens (*S. aureus, S.* Typhi*, E. coli*) and a group of plant pathogens (*B. nigrifluens, E. amylovora, S. marcescens, P. syringae*). Using disk diffusion assays at several concentrations, this work seeks to (i) characterize the inhibitory spectrum of *E. amoenum* extracts; (ii) determine whether the ethanol extract is more effective than the aqueous one; and (iii) identify bacterial species most and least sensitive. The findings will contribute valuable insight into the potential of *E. amoenum* as a natural antimicrobial source for both medical and agricultural applications.

Materials and Methods

A) Plant extraction: Aerial parts of *Echium amoenum* were shade-dried, powdered, and subjected to maceration. Thirty grams of the dried powder was mixed with 300 mL of distilled water or 96% ethanol (1:10 w/v) and kept on a rotary shaker at room temperature for 48 h. The mixtures were filtered through sterile Whatman No. 1 paper and centrifuged at 10,000 rpm for 10 min. The solvents were

evaporated, and the crude extracts were dried at 37 °C. Dried residues were scraped, transferred to sterile tubes, and stored at -22 °C until use (Ebrahimzadeh et al., 2008).

B) Preparation of dilutions: Stock solutions of aqueous and ethanolic extracts were prepared in dimethyl sulfoxide (DMSO). Final concentrations of 50, 100, and 200 mg/mL were obtained and stored at 4 °C until assays (Basri & Fan, 2005).

C) Bacterial strains: The bacterial strains used in this study included *S. aureus* (ATCC 25923), *S.* Typhi (ATCC 6539), *E. coli* (ATCC 25922), *B. nigrifluens* (ATCC 27627), *E. amylovora* (ATCC 15580), *S. marcescens* (ATCC 13880), and *P. syringae* (ATCC 19310). Fresh cultures were prepared on nutrient agar and incubated at 37 °C for 48 h.

D) Disk diffusion assay: Antibacterial activity was determined using the Kirby–Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines. Briefly, bacterial suspensions were adjusted to 0.5 McFarland standard and uniformly spread on nutrient agar using sterile swabs. Sterile blank disks were loaded with 20 µL of each extract dilution, dried under laminar flow, and placed on inoculated plates. Negative controls consisted of disks containing 20 µL of DMSO; streptomycin (10 µg/disk) was used as the positive control (Collins et al., 2010). Plates were incubated at 37 °C for 24 h. The diameters of inhibition zones were measured in millimeters using a ruler. All experiments were performed in triplicate (Figure 1).

E) Statistical analysis: Data were expressed as mean ± SD of three replicates. Differences among extract concentrations were analyzed using one-way ANOVA followed by Duncan's post-hoc test. Statistical significance was considered at $p < 0.05$. Analyses were performed using SPSS (version 27.0).

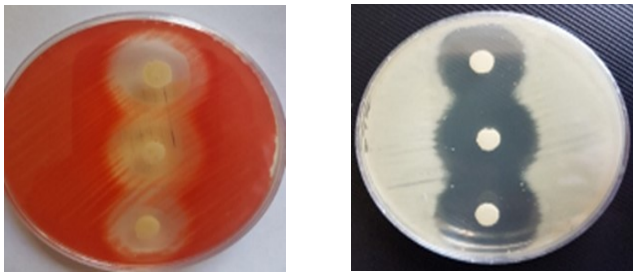

Fig 1. Inhibition zones formed by ethanolic extract of *Echium amoenum* against bacteria.

Results

The antibacterial activity of ethanolic and aqueous extracts of *Echium amoenum* was assessed against seven bacterial strains, including three human pathogens (*S. aureus, S.* Typhi, *E. coli*) and four plant pathogens (*B. nigrifluens, E. amylovora, S. marcescens, P. syringae*). Both extracts showed concentration-dependent effects, with inhibition zones increasing at higher concentrations.

A) Ethanolic extract: The ethanolic extract exhibited stronger antibacterial activity compared with the aqueous extract. At 50 mg/mL, inhibition zones ranged from 6.0 mm (*B. nigrifluens* and *E. coli*) to 14.3 mm (*S. marcescens*). With increasing concentration, inhibitory effects were markedly enhanced. At 200 mg/mL, inhibition zones reached 21.6 mm for *S. marcescens*, 19.3 mm for *E. amylovora*, and 17.6 mm for *S. aureus*, while *E. coli* and *B. nigrifluens* remained the least affected (12.6 mm and 7.6 mm, respectively) (Table 1).

Table 1. Mean inhibition zones (mm) of ethanolic *E. amoenum* extract.

Bacterium	50 mg/mL (mm)	100 mg/mL (mm)	200 mg/mL (mm)
B. nigrifluens	6	6.5	7.6
P. syringae	6	6.8	8.3
E. amylovora	13.6	17	19.3
S. marcescens	14.3	18.3	21.6
S. aureus	12.3	16.3	17.6
***S.* Typhi**	12	15	16.6
E. coli	6	8.3	12.6

B) Aqueous Extract: The aqueous extract displayed lower inhibitory activity, although it maintained a dose-dependent pattern. At 50 mg/mL, inhibition zones varied between 6.0 mm (*B. nigrifluens* and *E. coli*) and 10.6 mm (*S. aureus*). At 200 mg/mL, the extract produced inhibition zones of 14.6 mm against *E. amylovora* and 13.3 mm against *S. aureus*, while *B. nigrifluens* and *E. coli* were the least affected (7.0 mm and 10.6 mm, respectively) (Table 2).

Table 2. Mean inhibition zones (mm) of aqueous *E. amoenum* extract.

Bacterium	50 mg/mL (mm)	100 mg/mL (mm)	200 mg/mL (mm)
B. nigrifluens	6	6.5	7
P. syringae	6	6.5	7.3
E. amylovora	8.6	10.3	14.6
S. marcescens	6.6	8.3	12.3
S. aureus	10.6	11.3	13.3
***S.* Typhi**	7.6	9.6	12
E. coli	6	7.6	10.6

C) Effect of extract concentration: Statistical analysis using one-way ANOVA demonstrated significant differences in inhibition zones across the tested concentrations for both extracts ($p < 0.001$). Post-hoc Duncan's multiple range test confirmed that antibacterial activity increased significantly with higher concentrations, with the 200 mg/mL extract showing the greatest inhibitory effect against all tested strains. Overall, ethanolic extracts were significantly more effective than aqueous extracts across nearly all bacterial species tested ($p < 0.05$).

Discussion

The present study evaluated the antibacterial activity of ethanolic and aqueous extracts of *Echium amoenum* against seven bacterial strains, including human pathogens (*S. aureus*, *S.* Typhi, *E. coli*) and plant pathogens (*B. nigrifluens*, *E. amylovora*, *Serratia marcescens*, *P. syringae*). Our findings demonstrated a concentration-dependent inhibitory effect for both extracts, with the ethanolic extract exhibiting significantly stronger activity than the aqueous extract. These results are consistent with previous studies that have reported the antibacterial efficacy of *E. amoenum* extracts. For instance, Shariatifar et al. (2016) demonstrated significant antibacterial effects of *E. amoenum* extracts using various in vitro methods (15). Similarly, Kavehei et al. (2023) found that the ethanolic extract of *E. amoenum* exhibited antibacterial effects on *Staphylococcus aureus* and other bacterial strains (16). These studies corroborate our findings and underscore the potential of *E. amoenum* as a source of natural antimicrobial agents.

Our study demonstrated that both ethanolic and aqueous extracts exhibited dose-dependent antibacterial activity, with the 200 mg/mL concentration producing the largest inhibition zones for all tested bacterial strains. These findings are in agreement with previous reports indicating that ethanolic extracts of *Echium* species show superior antibacterial effects compared to aqueous and methanolic extracts (15).

In our study, plant pathogens such as *E. amylovora* and *S. marcescens* exhibited the highest susceptibility to both ethanolic and aqueous extracts, with inhibition zones reaching up to 21.6 mm at 200 mg/mL concentration. Conversely, *B. nigrifluens* and *E. coli* showed the least sensitivity, with inhibition zones of 7.6 mm and 12.6 mm, respectively, at the same concentration. This differential susceptibility is consistent with findings by Kavehei et al. (2023), who reported varying degrees of antimicrobial activity of

E. amoenum extracts against different bacterial species (15).

The antimicrobial activity of *E. amoenum* extracts can be attributed to the presence of bioactive compounds such as flavonoids, phenolic acids, and alkaloids. These compounds have been shown to possess antimicrobial properties by disrupting bacterial cell membranes, inhibiting enzyme activity, and interfering with DNA replication (17). The higher efficacy of the ethanolic extract may be due to its ability to solubilize a broader range of bioactive compounds compared to the aqueous extract, allowing for greater interaction with bacterial cells.

The significant antibacterial activity of *E. amoenum* extracts against both human and plant pathogens suggest their potential as natural alternatives to synthetic antibiotics and chemical bactericides. The dual applicability of these extracts in medical and agricultural fields is particularly valuable in the context of rising antimicrobial resistance and the need for environmentally friendly pest control methods.

Limitations and Future Research

While our study provides valuable insights into the antibacterial properties of *E. amoenum* extracts, several limitations should be considered. The in vitro nature of the study does not account for the complexities of in vivo interactions, and the specific mechanisms underlying the antimicrobial effects remain to be elucidated. Future research should focus on conducting in vivo studies to assess the efficacy and safety of *E. amoenum* extracts, as well as exploring their potential synergistic effects with other antimicrobial agents.

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Statements and Declarations

Conflicts of interest

The authors declare that there are no conflicts of interest.

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References

1. Palmer, G.H. and D.R. Call, Antimicrobial resistance: A global public health challenge requiring a global one health strategy. NAM Perspectives, 2013.
2. Ristaino, J.B., et al., The persistent threat of emerging plant disease pandemics to global food security. Proceedings of the National Academy of Sciences, 2021. 118(23): p. e2022239118.
3. El-Saadony, M.T., et al., Medicinal plants: bioactive compounds, biological activities, combating mult drug-resistant microorganisms, and human health benefits - a comprehensive review. Front Immunol, 2025. 16: p. 1491777.
4. Safaeian, L., et al., Cytoprotective and antioxidant effects of *Echium amoenum* anthocyanin-rich extract in human endothelial cells (HUVECs). Avicenna J Phytomed, 2015. 5(2): p. 157-66.
5. Naseri, N., K. Kalantar, and Z. Amirghofran, Anti-inflammatory activity of *Echium amoenum* extract on macrophages mediated by inhibition of inflammatory mediators and cytokines expression. Res Pharm Sci, 2018. 13(1): p. 73-81.
6. Nieto, L.G., et al., Estimated economic impact of fire blight on long-term orchard economic performance with susceptible and resistant rootstocks. Scientia Horticulturae, 2024. 337: p. 113478.
7. Zhao, Y.-q., et al., Fire blight disease, a fast-approaching threat to apple and pear production in China. Journal of Integrative Agriculture, 2019. 18 (4): p. 815-820.
8. Végh, A., et al., First Report of Bacterial Bark Canker of Walnut Caused by *Brenneria nigrifluens* in Hungary. Plant Dis, 2014. 98(7): p. 988.
9. Hasan, M.F., M.A. Islam, and B. Sikdar, First report of *Serratia marcescens* associated with black rot of Citrus sinensis fruit , and evaluation of its biological control measures in Bangladesh. F1000Res, 2020. 9: p. 1371.
10. Xin, X.-F., B. Kvitko, and S.Y. He, *Pseudomonas syringae*: what it takes to be a pathogen. Nature Reviews Microbiology, 2018. 16(5): p. 316-328.
11. Silva-Santana, G., *Staphylococcus aureus*: Dynamics of pathogenicity and antimicrobial-resistance in hospital and community environments - Comprehensive overview. Res Microbiol, 2025. 176(3-4): p. 104267.
12. Dougan, G. and S. Baker, *Salmonella enterica* serovar Typhi and the pathogenesis of typhoid fever. Annu Rev Microbiol, 2014. 68: p. 317-36.
13. Tchesnokova, V., et al., Gut resident *Escherichia coli* profile predicts the eighteen-month probability and antimicrobial susceptibility of urinary tract infections. medRxiv, 2024.
14. Larsson, D.G.J. and C.-F. Flach, Antibiotic resistance in the environment. Nature Reviews Microbiology, 2022. 20(5): p. 257-269.
15. Kavehei, M. and S.H. Sefidroo, Investigating the Antimicrobial Activity of Different Extracts of Echium on Selected Gram-Positive and Gram-Negative Bacteria. Tabari Biomedical Student Research Journal, 2023. 5(2): p. 18-24.
16. Shariatifar, N., A.E. Fathabad, and S. Madihi, Antibacterial activity of aqueous and ethanolic extracts of *Echium amoenum* on food-borne pathogens. Journal of Food Safety and Hygiene, 2016. 2(3/4): p. 63-66.
17. Patocka, J. and Z. Navratilova, Bioactivity of *Echium amoenum*: A mini review. Pharmacology, 2019. 20 (2): p. 14915-14917.