



Antibacterial activity of aqueous and ethanolic extracts of *Echium amoenum* on food-borne pathogens

Nabi Shariatifar^a, Ayub Ebadi Fathabad^{b*}, Soheila Madihi^a

^a Food Safety & Hygiene Division, Department of Environmental Health, Tehran University of Medical Sciences, Tehran, Iran

^b Department of Food Hygiene and Quality Control, Faculty of Veterinary Medicine, University of Urmia, Urmia, Iran.

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ABSTRACT

The antibacterial agents are a group of food additives that are used in food as preservative. The objective of this study was to determine the antibacterial activity of *Echium amoenum* flower using different in vitro methods. Having collected the flower of this plant in spring, ethanolic extracts of *Echium amoenum* were prepared in distilled water and absolute ethanol, respectively. Also, there are different concentration rates for the extract which was produced by microdilution Broth method in BHI medium and culturation in Mueller Hinton Agar medium. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) was determined with method of visual monitoring. The aqueous extracts (AEs) and ethanolic extracts (EEs) exhibited antimicrobial activity against Gram-positive and Gram-negative bacteria strains. The (AEs) and (EEs), at a concentration from 1250 to $16 \times 10^4 \mu\text{g/ml}$, showed a significant antibacterial effect expressed as minimum inhibitory concentration (MIC) against both Gram-negative and Gram-positive bacteria. *Staphylococcus aureus* and *Yersinia enterocolitica* were the strains more sensitive to the ethanol extract effect (MIC = 1250 $\mu\text{g/ml}$) and *Yersinia* for the ethanol extract (MIC = 2500 $\mu\text{g/ml}$). The data were expressed as the mean \pm the standard deviation and they were statistically analyzed by SPSS software using ANOVA. The findings indicated that the (AEs) of *Echium amoenum* flower can act as a natural antibacterial and as a possible food supplement or to be used in pharmaceutical industry after complementary tests.

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1. Introduction

Food-borne pathogens are a major health problem thus, the research for finding effective drugs against them is necessary. The Prevalence of food-borne disease caused by Food-borne pathogens has increased worldwide and is a major cause of mortality in people with immunodeficiency in developing countries (1-3). Therefore, immediate control of antimicrobial resistance by improved antibiotic use and reduce of infection, but the development of new antibiotics and natural antibiotics must be continued to maintain the

effectiveness of antimicrobial therapy of primary importance (4, 5). The World Health Organization (WHO) in developing countries estimates that about three quarters of the population relies on herbal medicines used in traditional medicine for primary health care as a basic human need. According to numerous studies, it has been found that some plants have antimicrobial activity and may be used to treat a variety of microbial diseases (6-8). *Echium amoenum*, famous as Borage is a large hairy annual herb that is a member of Boraginaceae family. It grows in most of Europe, in the Mediterranean region, as well as in the northern part of Iran (2, 9). Light blue flowers are star-shaped and fruit consists of four black brown nutlets. Borage blossom in

* Corresponding author. Tel.: +982188954914
E-mail address: ayubebadi1986@gmail.com

ordinary soil and may be divided into stems and cuts of the branches in sandy soil in a cold frame in the summer and autumn or reproduced from seed in good light soil from mid-March to May (2, 10). The flower and leave of borage is used as medicinal herb in France to treat of depression, stress and circulatory cardiovascular diseases, and as a poultice for inflammatory swellings, diuretic, a laxative, emollient, demulcent, and recently as a possible protective factor agent against cancer. (11, 12). Plant ingredients have been isolated by various researchers; they include gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), delta 6-fatty acyl desaturase, delta 8-sphingolipid desaturase, pyrrolizidine alkaloids, mucilage, resin, potassium nitrate and calcium salt combined with mineral acids (13, 14).

This study was designed to examine antibacterial activity of the (AEs) and (EEs) obtained from *Echium amoenum* flower against food-borne pathogens.

2. Materials and methods

2.1. Plant materials

The flower of *Echium amoenum* was collected from Karaj, Iran, during March 2016. The genus and plant species were approved by herbarium division of Department of Pharmacognosy, School of Pharmacy, Tehran University of Medical Sciences.

2.2. Preparation extracts

The aqueous extract (AEs) of the flower of *Echium amoenum* was obtained by adding 500ml of boiling water to 150 g of powdered flower material in a glass flask 1L and the ethanolic extract (EEs) of the flower of *Echium amoenum* was obtained by adding 500ml of 70% aqueous Ethanol to 150 g of powdered flower material in a glass flask 1L and both incubated at room temperature for 24 h on a rotating shaker (200 rpm). The extracts were filtered using Whatman No. 1 filter paper and then concentrated in vacuum at 40 °C using a rotary evaporator.

2.3. In vitro Antimicrobial activity test

2.3.1. Disk Diffusion Test

The aqueous extract and ethanolic extract were tested against *Staphylococcus aureus* (ATCC 25913), *Listeria monocytogenes* (ATCC 19117), *Escherichia coli* (ATCC 8739), *Yersinia enterocolitica* (ATCC 9610) and *Salmonella typhimurium* strain (ATCC 14028). The microorganisms were cultured in BHI (Brain Heart Infusion) for 18 hours at 37°C, and re-suspended in 0.5 Mac Farland Standard (5 x 10⁸ cfu/mL) and inoculated

directly in boards with Mueller-Hinton Agar (Merck). After the inoculation each micro-organism for determination of the antibacterial effect of extracts a disk diffusion method was used, ten micro-liter of each extract was putted on sterile paper disks (6 mm diameter). The system was incubated at 37°C/24 hours and then the halos of inhibition were measured (15, 16).

2.3.2. Micro dilution method

The extracts of *Echium amoenum* were diluted with Mueller Hinton Broth culture medium by using serial micro dilution method at a final concentration range from 32 to 0.25%. The antibacterial activity of extracts were evaluated in triplicate. Tests are performed under standard conditions for determine MIC and MBC values (15, 16).

2.4. Statistical analysis

Each experiment, from sample preparation to analysis, was repeated in triplicate, and the data were analyzed by SPSS software program and then analyzed by Excel software program version 2010.

3. Results

Aquatics extract and ethanolic extract of *Echium amoenum* are shown to exhibit widespread antimicrobial activity. Antibacterial susceptibility testing of extracts were performed by broth micro dilution.

The results of the antibacterial activities are presented in Table 1 all bacterial strains studied were inhibited by flowers of *Echium amoenum* extracts, with same degrees of inhibition. The MIC values of *Echium amoenum* ethanolic and aqueous extracts were as follows: *S.aureus* obtained MIC 1250 µg/ml and 4×10⁴ µg/ml, *L.monocytogenes* with MIC 2500 µg/ml and 8×10⁴ µg/ml, *Yersinia* with MIC 2.5×10³ µg/ml and 8×10⁴ µg/ml, *E. coli* with MIC 5×10³ µg/ml and 8×10⁴ µg/ml and *Salmonella* with MIC 5×10³ µg/ml and 16×10⁴ µg/ml.

The ethanolic and aqueous extracts of the flower of *Echium amoenum* showed antibacterial activity against all of the bacterial strains used in this study (*S.aureus*, with an average inhibitory zone of 11.5±0.87 mm and 11±0.85 mm, *E.coli*, with an average inhibitory zones of 1310.5±0.87mm and 12±1.21 mm, *L.monocytogenes* with an average inhibitory zone of 10 ±1.04 mm and 13±0.82 mm, *Y.enterocolitica* with an average inhibitory zone of 10±1.22 mm and 13.5±1.04 mm and *S.typhimurium* with an average inhibitory

Table 1. Determination of MIC and MBC value ($\mu\text{g/ml}$) for water and ethanol extract berberis against pathogenic bacterial strains

Test	Staphylococcus aureus		Listeria monocytogenes		Yersinia enterocolitica		Escherichia coli		Salmonella typhimurium	
	aqueous	ethanol	aqueous	ethanol	aqueous	ethanol	aqueous	ethanol	aqueous	ethanol
MIC ($\mu\text{g/ml}$)	4×10^4	1.25×10^3	8×10^4	1.25×10^3	8×10^4	2.5×10^3	8×10^4	5×10^3	16×10^4	5×10^3
MBC ($\mu\text{g/ml}$)	8×10^4	2.5×10^3	16×10^4	1.25×10^3	16×10^4	2.5×10^3	16×10^4	5×10^3	16×10^4	10^4

zone of 9 ± 0.87 mm and 11.5 ± 0.87 mm) (Table 2,3) ($P < 0.05$).

Table 2. Comparison of average inhibitory halo diameter (mm) of various bacterial strains for ethanol extract

Bacterial strain	Samples	Min	Max	Average \pm SD
S. aureus	3	11	12.5	11.5 ± 0.87^a
E. coli	3	9.5	11.5	10.5 ± 0.87^a
L.monocytogenes	3	9	11.5	10 ± 1.04^a
Y.enterocolitica	3	9.5	11.5	10 ± 1.22^a
S. typhimurium	3	8.5	10	9 ± 0.87^a

Table 3. Comparison of average inhibitory halo diameter (mm) of various bacterial strains for aqueous extract

Bacterial strain	Samples	Min	Max	Average \pm SD
S. aureus	3	10.5	12.5	11 ± 0.85^b
E. coli	3	11.5	13	12 ± 1.21^b
L.monocytogenes	3	12	13.5	13 ± 0.82^b
Y.enterocolitica	3	12.5	14.5	13.5 ± 1.04^b
S. typhimurium	3	10.5	12	11.5 ± 0.87^b

4. Discussion

Our results showed that the aqueous and ethanolic extracts at higher concentrations of Echinium amoenum increases the antibacterial effect. Antimicrobial susceptibility was studied using classical microbiological techniques with disk diffusion, MIC and MBC determination. Abolhassani (2004) showed concentration-dependent antibacterial activity for Echinium amoenum Flower aqueous extract against Staphylococcus aureus, and it is believed that borage flower can be used for controlling infectious and fever (17). Farahani (2013) reported Echinium amoenum extract exhibited significant antiviral activity at nontoxic concentrations to the cell line used and herbal extract was inhibitor of virus replication at concentrations higher than $400 \mu\text{g/ml}$ (18). Semnani et

al. (2009) showed antimicrobial activity of Echinium italicum oil using disk diffusion method and determination of minimal inhibitory concentration values against Bacillus subtilis PTCC 1023, Staphylococcus aureus PTCC 1112, Escherichia coli PTCC 1330, Salmonella typhi PTCC 1639, Pseudomonas aeruginosa PTCC 1074, Aspergillus niger PTCC 5011 and Candida albicans PTCC 5027. Echinium italicum essential oil exhibited concentration dependent antibacterial activity against all the tested microorganisms (6). Sabour et al (2014) reported Echinium amoenum methanolic extract exhibited significant antiviral activity using disk diffusion method and determination of minimal inhibitory concentration values against Acinetobacter baumannii (19). Saberifard et al (2014) showed antimicrobial activity of Echinium amoenum extracts using agar a disk diffusion method and determination of minimal inhibitory concentration values against Staphylococcus aureus ATCC 1112, Escherichia coli ATCC 25922 and Pseudomonas aeruginosa ATCC 27853 (20). Herbal extracts as well as essential oils have antimicrobial effects, like garlic essential oil effects on Coliforms, Staphylococcus aureus and Psychrotrophic bacteria (21). Finally, we conclude that most of the results of this study are in good agreement with the traditional uses of the investigated plant. Ethanolic extracts exhibited a significant antibacterial activity.

5. Conclusion

In this study, the antimicrobial activity of aqueous and ethanolic extracts of Echinium amoenum were performed against food-borne pathogens. The plant ethanolic extract may be effective against other gram-positive and gram-negative bacteria. Echinium amoenum extracts can be useful as replacement of artificial preservatives that often are used in food, due to this natural preservatives have shown antibacterial activity.

Conflict of interest

The authors have no conflict of interest.

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None.

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