Tabari Biomedical Student Research Journal

OPEN ACCESS

ORIGINAL: Investigating the Antimicrobial Activity of Different Extracts of *Echium* on Selected Gram-Positive and Gram-Negative Bacteria

Masoud Kavehei | Department of Biology, School of Basic Sciences, Islamic Azad University, Borujerd Branch, Borujerd, Iran
Seyed Hasan Sefidroo | Department of Biology, School of Sciences, University of Guilan, Rasht, Iran

ARTICLE INFO

Submitted: 15 May 2023
Accepted: 19 Aug 2023
Published: 01 Sep 2023

Keywords:
Antimicrobial activity;
Echium amoenum;
Gram negative bacteria;
Gram positive bacteria

Correspondence:
Seyed Hasan Sefidroo, Department of Biology, School of Sciences, University of Guilan, Rasht, Iran.
Email:
armansefidroo9@gmail.com
ORCID: 0009-0008-9893-9185

Citation:
Kavehei M, Sefidroo SH. Investigating the Antimicrobial Activity of Different Extracts of Echium on Selected Gram-Positive and Gram-Negative Bacteria. Tabari Biomed Stu Res J. 2023;5(2):18-24.

doi 10.32598/tbsrj.v5i2.10532

ABSTRACT

Introduction: Medicinal plants have had a special value and importance in providing health and wellness of communities both in terms of treatment and prevention of diseases. The aim of this research was to investigate the antimicrobial activity of different extracts (aqueous, ethanolic, and methanolic) of two types of *Echium* (*Helioteropium ellipticum* and *Anchusa strigosa*) on *Staphylococcus aureus*, *Bacillus Cereus*, *Escherichia Coli*, *Salmonella Typhi* and *Shigella* dysentery bacteria.

Material and Methods: Extracts were prepared by soaking method. Antimicrobial activity was determined by two methods: dilution sequence in liquid medium and diffusion from disk in solid medium. The data were analyzed with SPSS24 software.

Results: Gram-positive bacteria showed more sensitivity to the extracts than Gram-negative bacteria. Antimicrobial activity of *Heliotropium ellipticum* was more than *Anchusa strigosa*. Aqueous extract has the lowest and ethanolic extract has the highest antimicrobial activity. *Staphylococcus aureus* showed the highest and *Escherichia coli* the lowest sensitivity to the extracts. *Staphylococcus aureus* bacteria showed the largest diameter of non-growth aura (20.67 mm) at the concentration of 50 mg/ml of ethanolic extract, and the MIC of *Staphylococcus aureus* had the highest sensitivity at 312.5 PPM dilution.

Conclusion: The two tested species have good antimicrobial activity and they can be used as natural substitutes for chemical antimicrobial agents.

Introduction

Echium amoenum or snake plant (scientific name: *Echium*) is the name of a genus of Borage family. This plant has many healing properties. The *Boraginaceae* family includes 95 genera and more than 1600 species. The plants of this family grow in all temperate and warm regions, especially in the Mediterranean regions, and are very rare in the arctic regions. Some plants of this family are herbaceous, such as *Echium amoenum*. The plants of this family have no edible or industrial use and instead have medicinal properties and almost all of them are glazed and contain sodium nitrate and are considered mild diuretics. Flowers, stems and roots of most of the plants of this family have a therapeutic effect (1-4). One of the local uses of *Echium amoenum* is for colds, anti-depressants, anti-stress, fever reducers, diuretics, disinfectants, and sedative properties (5-7).

Infectious diseases are one of the most common diseases in the world that impose a great financial burden on human societies.

Although synthetic anti-biotics have been able to play an important role in the treatment of infectious diseases in the past decades, they have also had problems (8). As microorganisms become more resistant to antibiotic compounds, herbal antimicrobials are an interesting source of new active ingredients. It has been proven that the use of dried plant extracts strengthens the therapeutic effect and prevents side effects due to the accumulation of substances in the plant. These extracts are available in various medicinal forms such as tablets, capsules, ointments, syrups, etc., which are desirable for the people of the 21st century (1). Therefore, the search for alternative anti-microbial agents is necessary. One of the possible strategies for this goal includes identifying and investigating the properties of bioactive plant materials that have antimicrobial activity (9). In one study shows that the aqueous extract of *E. amoenum* has significant antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Pseudomonas aeruginosa*. Aqueous extract of *E. amoenum* from dried flowers also showed concentration-dependent antibacterial activity against *Staphylococcus aureus* 10). The traditional use of Iranian borage flower for infectious diseases and fever control seems justified. Antibacterial activity of *E. amoenum* plant against *Staphylococcus aureus 8327* was evaluated by agar well diffusion, disk diffusion and minimum inhibitory concentration (MIC) methods. The MIC of this extract was equal to 6.2 µg/ml and the results confirm the concentration-dependent antibacterial activity of this plant extract against the studied bacteria (11). In another study, Bonjar et al evaluated the antibacterial activity of the methanol extract of E. amoenum against Micrococcus *luteus*, *Serratia marcescens*, *Klebsiella pneumonia* and *Bordetella bronchiseptica* using disk diffusion and MIC methods. The results showed that this extract is effective against B. bronchiseptica with an MIC of 15 mg/ml. In another study, the trypanocidal activity of *E. amoenum* against *Trypanosoma cruzi* was investigated and the minimum lethal concentration was calculated. The results showed that different extracts of *E. amoenum* did not show significant activity against *T. cruzi* epimastigotes (12). However, most of the plant species in Iran have not been widely investigated for antibacterial activities. The purpose of this research is to investigate the antimicrobial effect of various extracts of the *E. amoenum* species on gram-positive and gram-negative bacteria.

Methods

Collecting two types of *E. amoenum*

The tested species of E.amoenum flower (*Helioteropium ellipticum* and *Anchusa strigosa*) were collected in the flowering stage in the summer of 2015 from the foothills of Delfan city located in the northwestern part of Lorestan province. Then, both species were completely dried in the shade, and then some of the dried plant was made into powder in a Chinese mortar and 25 grams of the powder was poured into closed containers (plastic-glass). Then it was placed in a 70-degree bain marie for one hour, then in the refrigerator for one hour, and then at the laboratory temperature for 22 hours, and this was repeated for three days.

Preparation of extracts

The soaking method was used to prepare the extract. 25 g of plant powder was mixed with 100 ml of solvent and after 48 hours of storage at ambient temperature, the mixture was filtered with Whatman No. 1 filter paper and dried at 40°C in an oven, then different concentrations were prepared and kept at refrigerator temperature until the start of microbial tests (1). To prepare different dilutions of the extracts, first 20% DMSO (20% DMSO and 80% sterile distilled water) was prepared and 3 concentrations of the extracts (10, 25 and 50 mg/ml) were prepared.

Microorganisms

In this study, the common human pathogenic bacteria (gram-positive and gram-negative) were used; including *Staphylococcus aureus*,

[DOI: 10.32598/tbsrj.v5i2.10532]

Bacillus cereus, *Escherichia coli*, *Salmonella typhi*, and *Shigella dysenteriae*, which were obtained from university centers.

Preparation of microbial suspension

Three milliliters of sterile saline (9 g of pure NaCl in 1 liter of distilled water) were poured into the cuvette of the device. Then, with a sterile swab, put some of the fresh bacterial culture (18-24 hours ago) in the saline inside the cuvette and make it completely uniform. So that the absorbance of this suspension containing bacteria is 0.1-0.115 at the wavelength of 600 nm. This suspension contains about 108 bacteria per milliliter (CFU/ml) (7).

Preparation of microbial culture medium

Mueller Hinton culture medium was used for bacterial culture. To prepare Muller's Hinton Broth medium, first 1/2 gram of this medium was poured into 100 ml of distilled water and melted in a microwave oven and then sterilized in an autoclave at 121°C for 15 minutes. During use, its temperature was lowered to below 40˚C. To prepare Mueller Hinton agar culture medium, 1.5 grams of agar was added for every 100 ml of distilled water and the previous steps were performed.

Determining the antimicrobial activity of Borage plant extracts in solid medium (disc diffusion)

After melting the culture medium and bringing its temperature to about 40˚C, the culture medium was poured into plates with a diameter of 10 cm. Then 100 µl of the microbial suspension were poured on the culture medium and spread over the surface of the culture medium with a completely sterile swab. Then, the blank paper disc purchased from Padten Teb Company was placed on the culture medium and 15 µl of the concentration prepared from the extract was poured on the disc. The bacterial medium was placed in a greenhouse at 37˚C for 24 hours. Then, the diameter of the inhibition aura was measured with a ruler. Finally, it was compared with positive control (tetracycline antibiotic) and negative control (solvent) (8).

Determination of MBC and MIC

To determine MIC (minimum inhibitory concentration) and MBC (minimum bactericidal concentration), dilution sequence method was used in 96-well microplate. First, 150 µl of Mueller Hinton Broth culture medium were poured into all the wells. Then, in the first well, 150 microliters of the concentration of 80 mg/ml of the extract were poured, and after mixing, 150 µl were removed and added to the second well. This was done to the last well and the last 150 µl was discarded. Then, 10 µl of microbial suspension were added to each well. Greenhouse planting was done like the disk diffusion method. After the end of the observation wells greenhouse time, the last well that did not have turbidity caused by the growth of microbes was introduced as MIC. To determine the MBC of the contents of the well, which was considered as MIC. Two wells that were cultivated in advance and placed in the greenhouse in the right environment, temperature and time. The concentration of the well in which the target microbe did not grow was considered MBC (9).

Statistical analysis

The experiment was conducted in the form of a random design with three repetitions, then the laboratory data was collected and entered into the computer. The normality of the data was ensured. Then, analysis was done using ANOVA test with via of SPSS 24 software with alpha 0.05.

Results

Determination of MIC, MBC of extracts

In ***Table 1***, gram-positive bacteria have lower MIC and MBC than gram-negative bacteria, and in other words, they are more sensitive to the antimicrobial activity of extracts. So that *Staphylococcus aureus* had the lowest and *Escherichia coli* the highest amounts. Regarding the type of extracts, the polar extract (blue) produced more MIC and

[DOI: 10.32598/tbsrj.v5i2.10532]

MBC, and in other words, they showed less antimicrobial activity than the ethanolic and methanolic extracts.

The effect of aqueous, ethanolic and methanolic extracts of *Echium amoenum* (*Anchusa strigosa* and *Helioteropium ellipticum*) on the studied bacteria

Table 2 and ***3*** examine the aura of non-growth in the studied samples in three replications with different concentrations of *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Salmonella Typhi* and *Shigella dysenteriae* bacteria. The diameter of the aura of non-growth at the concentration of 50 mg/ml is greater than the lower dilutions in all three types of extracts. Also, the diameter of the non-growth aura of the positive control is greater in all three dilutions than all three types of extracts.

Table 1. The results of determining MIC and MBC (concentration in ppm)

Bacteria	*Anchusa strigosa*						*Helioteropium ellipticum*					
	Aqueous extract		Ethanolic extract		Methanolic extract		Aqueous extract		Ethanolic extract		Methanolic extract	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Staphylococcus aureus	5000	5000	625	625	2500	5000	2500	5000	312.5	312.5	312.5	625
Bacillus cereus	5000	10000	625	1250	5000	5000	5000	5000	625	625	312.5	625
Escherichia coli	20000	40000	2500	5000	10000	20000	20000	20000	5000	2500	2500	5000
Salmonella typhi	5000	10000	1250	2500	10000	10000	5000	10000	1250	1250	625	1250
Shigella dysentery	20000	20000	2500	5000	20000	20000	20000	20000	2500	2500	1250	2500

Table 2. The effect of aqueous, ethanolic and methanolic extracts of *Echium amoenum* (*Anchusa strigosa* and *Helioteropium ellipticum*) on the studied bacteria

Bacteria	Extract	Aqueous extract in three dilutions			Ethanolic extract in three dilutions			Methanolic extract in three dilutions			Control
		10 mg	25 mg	50 mg	10 mg	25 mg	50 mg	10 mg	25 mg	50 mg	
Staphylococcus aureus	*Anchusa strigose*	9	9.6	13	9	10	17	9	10	15	22
	Helioteropium ellipticum	11.6	13	15.6	16	17	20.6	12.7	14	17	
Bacillus cereus	*Anchusa strigose*	7.67	10.6	14.3	8.3	11.6	16.7	7.3	11	14	18
	Helioteropium ellipticum	10.6	12	14	12	14	17	11.3	11	16.6	
Escherichia coli	*Anchusa strigose*	6	7	11	7	7.6	14	7	7	12	17
	Helioteropium ellipticum	6	7	11.6	7.3	8.3	15	6.3	8	14	
Salmonella typhi	*Anchusa strigose*	7.3	9	11.6	9.6	10	12.3	7.3	8	12	18
	Helioteropium ellipticum	8	10	12	8.3	11	14.6	8	12	14	
Shigella dysentery	*Anchusa strigose*	7.3	7	11	7	7.6	14.6	7	7	14	16
	Helioteropium ellipticum	7.3	8	10	8.3	11	15	10.6	13	14	

Discussion

For about two decades, the use of antimicrobial properties of plants in the pharmaceutical, agricultural and food industries has been growing day by day. According to the results of this research, in general, the antimicrobial properties of *H. ellipticum* species are more than *A. strigosa* species, which could be due to various reasons. Climatic and weather conditions (precipitation rate, altitude above sea level, soil type, temperature and environmental stresses), genus, species and subspecies of medicinal plant and the way of maintaining medicinal plant cause destructive physiological changes in the plant. And this affects the secondary and effective compounds in medicinal plants. The stages of plant growth also have a great impact on the antimicrobial activity of medicinal plants. The antimicrobial compounds of these plants are strongly influenced by the growth stage of the plant, so that generally the most antimicrobial activity of medicinal plants is related to their flowering stage. The extraction method is also one of the other things that affect the antimicrobial activity of medicinal plants (13, 14). Therefore, it is

[DOI: 10.32598/tbsrj.v5i2.10532]

[DOI: 10.32598/tbsrj.v5i2.10532]

obvious and acceptable that different species have different effective compounds and, as a result, different antimicrobial activity, and in almost all the researches conducted in this field, results similar to this research have been obtained. In a study conducted by Bagci on the antimicrobial activity of two species of *Tanacetum balsamita* plants, it was shown that these two species have different antimicrobial compounds and therefore different antimicrobial activity, which is in accordance with the results of this research (15, 16). Also, the results of this research are consistent with the results of Kotan, who investigated the antimicrobial activity of different extracts of several medicinal plants (9, 17). In the conducted research, there is a significant difference between the antimicrobial activity of the extracts. So that aqueous extract showed the least and ethanolic extract showed the most antimicrobial activity. The antimicrobial compounds of medicinal plants are very diverse, among these compounds, terpenes, terpenoids, and glucosinolates can be mentioned. Each of the compounds in essential oils and extracts of medicinal plants have a certain antimicrobial activity, which of course is subject to several factors. According to the results of similar research conducted in the past, it is clear that the major antimicrobial activity of medicinal plants is related to hydrophobic compounds such as monoterpenes. The higher the amount of these compounds in the extract of medicinal plants, the more antimicrobial activity they have (18). Water is a polar compound and solvent that can dissolve a large number of polar substances in itself, and on the contrary, it does not have much ability to dissolve non-polar and hydrophobic compounds, the opposite is true for ethanol and methanol. For this reason, the hydrophobic compounds of medicinal plants that have a lot of antimicrobial activity are dissolved in less amount in water and are mostly extracted by non-polar solvents. Therefore, the lower antimicrobial activity of the aqueous extract of the investigated species compared to the other two extracts is mentioned for this reason. Shataye performed the antimicrobial activity of the aqueous and ethanolic extracts of the roots of Anchusa strigosa by disc diffusion method on a number of Gram-positive and Gram-negative bacteria. The results showed that the antimicrobial activity of the ethanolic extract was higher than that of the aqueous extract, and *Staphylococcus* bacteria was the most sensitive bacterium to the components of the ethanolic extract. These results are in accordance with the results of this research. In the research that was conducted on two Gram-positive bacteria (*Staphylococcus aureus*, *Bacillus cereus*) and three Gram-negative bacteria (*Escherichia coli*, *Salmonella typhi*, and *Shigella dysenteriae*), it was found that the microbes showed different resistance and sensitivity to the extracts. In general, gram-positive bacteria were less resistant than gram-negative bacteria. Generally, *Staphylococcus aureus* had the least resistance and *Escherichia coli* the most resistance to the antimicrobial compounds of different extracts. Bacteria show a wide range of resistance or sensitivity to the compounds of essential oils and extracts of medicinal plants. Gram-positive bacteria generally have a thick Peptidoglycan wall around them. Although this wall is thick, it does not have much resistance to the passage of medicinal plant compounds, and for this reason, these compounds easily enter the bacterial cell and cause the death of the bacteria by disrupting its various parts (disruption and disintegration of the membrane, release of cytoplasmic contents, and disruption of the work of mitochondria and...). Gram-negative bacteria have a lipopolysaccharide membrane. This membrane shows a significant resistance against the penetration of medicinal plant compounds, and therefore, in general, gram-negative bacteria show more resistance to the essence and extracts of medicinal plants (18). According to the results of Desiree et al., the results in similar research also obtained similar results with this research (7). Also, in one research, which examined the extract from the medicinal plant of *Echium amoenum* and investigated its antimicrobial effect, the result showed that the antibacterial effect on bacteria such as micrococci is significant. The results of the experiment showed that the aqueous-alcoholic extract of burdock leaves has significant antimicrobial effects on gram-positive micrococcus bacteria, while it has no significant effect on *Escherichia coli* bacteria. The results of this study agree with the results of our study that the ethanolic extract of *Echium amoenum* has an antibacterial effect on *Staphylococcus aureus* (19). In this study, three concentrations (10, 25 and 50 mg/ml) were used. The obtained results showed that there is a significant difference between the antimicrobial activity of the concentrations. With the increase in concentration, the antimicrobial activity of the extracts and the diameter of the aura of non-growth also increased, which is due to the penetration of larger amounts of extract compounds into the bacterial cell and, as a result, more effect. Jabrian and his colleagues investigated the antimicrobial effect of different concentrations of several extracts of *Falcaria vulgaris* plant on gram-negative and gram-positive bacteria. The results, which are completely consistent with the results of this research, showed that the antimicrobial activity of the extracts increases with the increase in concentration. Also, the anti-microbial activity in liquid and solid environment, which was determined by disk diffusion method and dilution sequence, respectively, was consistent. So that an extract that has a higher antimicrobial activity in a solid environment also has approximately the same effect in a liquid environment compared to other extracts (20). In one research also evaluated the antibacterial, antifungal and antioxidant activity of the extracts of *Echium amoenum* and *Marrubium anisodan* plants. The results showed that the methanolic extract of the root of the borage had the highest inhibition on the gram-positive bacteria *Staphylococcus aureus* and *Bacillus subtilis*. Considering the importance of flavonoid compounds in treating human diseases and preventing lipid oxidation in food, the high number of flavonoids in the leaves of *Echium amoenum* plant and Sugarcane plant is significant. These results are consistent with the results of our research in which increasing the concentration of the extract led to the inhibition of *Staphylococcus aureus* and *Bacillus cereus* bacteria (21).

Conclusion

In general, it can be concluded that the two tested species have good antimicrobial activity. As a result, the extracts of these plants can be used as natural antimicrobial substances in the pharmaceutical, food, agricultural industry or the production of cosmetics.

Ethical standards statement

All of the protocols in this study were reviewed and approved by the Research Ethics Committee of Guilan University.

Conflicts of interest

The authors declare no conflict of interest.

Authors' contributions

Each author has made an important scientific contribution to the study and has assisted with the drafting or revising of the manuscript.

References

1. Costion CM, Lorence DH. The endemic plants of Micronesia: A geographical checklist and commentary. Micronesica. 2012;43:51-100.
2. Aouadi K, Hajlaoui H, Arraouadi S, Ghannay S, Snoussi M, Kadri A. Phytochemical Profiling, Antimicrobial and α-Glucosidase Inhibitory Potential of Phenolic-Enriched Extracts of the Aerial Parts from Echium humile Desf.: In Vitro Combined with In Silico Approach. Plants. 2022;11(9):1131.
3. Jin J, Boersch M, Nagarajan A, Davey AK, Zunk M. Antioxidant properties and reported ethnomedicinal use of the genus Echium (Boraginaceae). Antioxidants. 2020; 9(8):722.

[DOI: 10.32598/tbsrj.v5i2.10532]

4. Kefi S, Essid R, Mkadmini K, Kefi A, Haddada FM, Tabbene O, Limam F. Phytochemical investigation and biological activities of Echium arenarium (Guss) extracts. Microbial pathogenesis. 2018;118: 202-10.
5. Yadav S, Sharma A, Nayik GA, Cooper R, Bhardwaj G, Sohal HS, Mutreja V, Kaur R, Areche FO, AlOudat M, Shaikh AM. Review of shikonin and derivatives: isolation, chemistry, biosynthesis, pharmacology and toxicology. Frontiers in Pharmacology. 2022; 13:905755.
6. Abolhassani M. Antibacterial effect of borage (Echium amoenum) on Staphylococcus aureus. Brazilian Journal of infectious diseases. 2004;8:382-5.
7. Désirée CKR, René FKP, Jonas K, Bibiane DT, Roger SM, Lazare K. Antibacterial and Antifungal Activity of the Essential Oil Extracted by Hydro-Distillation from" Artemisia annua" Grown in West-Cameroon. British journal of pharmacology and toxicology. 2013;4(3):89-94.
8. Alade P, Irobi O. Antimicrobial activities of crude leaf extracts of Acalypha wilkesiana. Journal of ethnopharmacology. 1993;39(3):171-4.
9. Kotan R, Dadasoğlu F, Karagoz K, Cakir A, Ozer H, Kordali S, et al. Antibacterial activity of the essential oil and extracts of Satureja hortensis against plant pathogenic bacteria and their potential use as seed disinfectants. Scientia horticulturae. 2013;153:34-41
10. Shariatifar N, Fathabad AE, Madihi S. Antibacterial activity of aqueous and ethanolic extracts of Echium amoenum on food-borne pathogens. Journal of Food Safety and Hygiene. 2016;2(3/4):63-6.
11. Abolhassani M. Antiviral activity of borage (Echium amoenum). Archives of Medical Science. 2010;6(3):366-9.
12. Azizi H, Ghafari S, Ghods R, Shojaii A, Salmanian M, Ghafarzadeh J. A review study on pharmacological activities, chemical constituents, and traditional uses of Echium amoenum. Pharmacognosy Reviews. 2018; 12(24).
13. Kamazeri TSAT, Samah OA, Taher M, Susanti D, Qaralleh H. Antimicrobial activity and essential oils of Curcuma aeruginosa, Curcuma mangga, and Zingiber cassumunar from Malaysia. Asian Pacific journal of tropical medicine. 2012;5(3):202-9.
14. Rios J-L, Recio MC. Medicinal plants and antimicrobial activity. Journal of ethnopharmacology. 2005;100(1-2):80-4.
15. Bagci E, Kursat M, Kocak A, Gur S. Composition and antimicrobial activity of the essential oils of Tanacetum balsamita L. subsp. balsamita and T. chiliophyllum (Fisch. et Mey.) Schultz Bip. var. chiliophyllum (Asteraceae) from Turkey. Journal of Essential Oil Bearing Plants. 2008;11(5):476-84.
16. Smith-Palmer A, Stewart J, Fyfe L. The potential application of plant essential oils as natural food preservatives in soft cheese. Food microbiology. 2001;18(4):463-70.
17. Delaquis P. Antimicrobial properties of isothiocyanates in food preservation. Food Technol. 1995;49:73-84.
18. Burt S. Essential oils: their antibacterial properties and potential applications in foods—a review. International journal of food microbiology. 2004;94(3):223-53.
19. Asadollahi F, Mehrzad J, Chaichi MJ, Razavizadeh AT. Photoimmunological properties of borage in bovine neutrophil in vitro model. Journal of Photochemistry and Photobiology B: Biology. 2015;151:270-5.
20. Jaberian H, Piri K, Nazari J. Phytochemical composition and in vitro antimicrobial and antioxidant activities of some medicinal plants. Food chemistry. 2013;136(1)237-44.
21. Shurovi FS. Determination of Antioxidant and Antimicrobial Activity of Dichloromethane Extract of Garcinia cowa stem: East West University; 2017.

[DOI: 10.32598/tbsrj.v5i2.10532]