

Original Article

Evaluation of the Antibacterial Effect of *Echium Amoenum* Fisch. ET MEY. against Multidrug Resistant *Acinetobacter Baumannii* Strains Isolated from Burn Wound Infection

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Abstract

Background: *Acinetobacter baumannii* in recent decay has become a concern in hospitals for its ability to acquire antibiotic resistance determinants rapidly and becoming resistant to almost all antibiotic classes. Borage (*Echium amoenum* Fisch. et Mey.), is a wild annual plant of Boraginaceae family, grows in northern mountains of Iran and has largely been used by Iranian folk as a mood enhancer, anti-anxiolytic, anti-inflammatory, a laxative, an emollients and also it has been used for treatment of infectious diseases. So, in this study the methanolic extract of dried flowers of *Echium amoenum* were tested against the isolates of *Acinetobacter baumannii* from wound of burn patients.

Methods: 30 drug resistant *Acinetobacter baumannii* strains which were isolated from burn wounds at the Motahari hospital of Tehran, Iran, were selected. Antibacterial activity of the methanolic extract was evaluated by the disc diffusion method based on CLSI protocol 2012.

Results: The mean diameter of the inhibition zone for different extracts were; 9.967±6.139 mm at the concentration of 4000 ppm, at the concentration of 400 ppm 13.37±5.45 mm, 13.53±5.49 mm at the concentration of 200 ppm, 14.77±5.17 mm at the concentration of 100 ppm and 14.13±5.7806 mm at the concentration of 50 ppm.

Conclusion: Clinical strains of the *A. baumannii* were almost highly resistant to imipenem which is the common choice of antibiotic therapy in the hospitals. Due to the calculated p value≤0.05 in this study, it can say that borage extract can be as good as or even better than the imipenem which is used in the hospitals now. Doing other in vitro and in vivo studies are recommended in further studies.

Keywords: Borage, *Echium amoenum* Fisch. Et Mey, *Acinetobacter baumannii*, antibacterial sensitivity test, burn wound infection, multi drug resistant

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Introduction

In the recent decades emerge of antibiotic resistant pathogens has been a worldwide problem. Also the undesirable side effects of some of the antibiotics made us to search for new sources to combat these problems¹. *Acinetobacter baumannii* (*A. baumannii*) is a nonfermentative, gram-negative, nonmotile, oxidase-negative bacillus. This pathogen rapidly emerges in health care units and becomes an important nosocomial infection². *A.baumannii* can cause bacteremia, pneumonia, meningitis, urinary tract infection, and wound infection³. Its ability to survive under wide range of environments has made it a successful endemic health care-associated pathogen⁴. *A.baumannii* has a remarkable ability to rapidly become resistant to many classes of antibiotics. So this pathogen from past two decades was multi drug resistance (MDR)^{5,6}. It is considered that resistance against carbapenems is adequate enough to define as highly resistant bacteria^{7,8}. Search for new materials to fight against this problem seems necessary. On the other hand, the potential of traditional herbal medicines cannot be underestimated. Antibacterial properties have been reported to be found in the wide range of medicinal plants⁹. *Echium amoenum* is a large, hairy, annual plant that is a member of *Boraginaceae* family¹⁰. This plant grows in northern mountains of Iran, western Mediterranean region, Spain, North Africa and hot regions of Europe¹¹. The flowers are light blue and like as star and the fruit consists of four brownish-black nutlets. Borage flourishes in the ordinary soil and may be propagated by division of rootstocks and by cuttings of shoots in sandy soil in a cold frame in summer and autumn or from seeds grown in good light soil from mid of March to May¹². It has been used by Iranian folk as a mood enhancer, an anxiolytic, anti inflammatory, anti laxative, an emollients and also it has been used for treatment of infectious diseases^{13,14}. The antiviral properties of *Echium amoenum* and also its antibacterial effect against *Staphylococcus aureus* have been proven¹³⁻¹⁵. In this study we evaluated the methanolic extract of *Echium amoenum* for its antibacterial properties on *Acinetobacter baumannii* strains which were isolated from clinical samples of

burn wound infection.

Methods

Plant collection and extraction process: The flowers of *Echium amoenum* were purchased from a well-known grocery of Tehran. At first, the flowers were washed and ground in to fine powder. Then, the powder were poured in the percolator apparatus and covered by methanol and remained for three days to be soaked in methanol. Then the methanolic extract was gathered, filtered and freeze dried for three hours.

Survey of the antibacterial effect of methanolic extract: 30 drug resistant *Acinetobacter baumannii* strains which were isolated from burn wounds at the Motahari hospital of Tehran, Iran, were selected during 2013. Antibacterial activity of the methanolic extract was evaluated by the disc diffusion method based on CLSI protocol 2012¹⁶. At first, the 40 mg of the dried extract were weighted, dissolve it in 10 ml of 10% DMSO and were filtered it by 0.45 micrometer filter to prepare the concentration of 4000 ppm. Then, further 2 times dilutions were prepared and the concentration of 400, 200, 100 and 50 ppm were made. The sterile blank discs were soaked for 12 hours in the prepared solutions and let to be dried under the safety cabinet for 2 hours. The DMSO 10% soaked disks and distilled water impregnated discs were prepared as control disks, simultaneously. Consequently, the bacteria suspensions which were equal to the 0.5 of McFarland turbidity (1.5×10^8 cfu/ml) were prepared of each isolates. Then by a sterile swab, bacterial cultivation was done on the Mueller-Hinton agar and the prepared discs of the extract, DMSO 10%, distilled water and the antibiotic disks of the imipenem, ciprofloxacin and colistin were put on the medium. All plates were incubated at 37°C for 24 hours. After that time the diameter zone of inhibition was determined. The results were analyzed by STAT software and calculation the multiple t-test. Also, the 6th graph pad prism was uses for drawing of the graphs.

Results

In this descriptive study, we evaluated the inhibition zone of the methanolic extract of *Echium amoenum* on the clinical multi drug resistance of *Acinetobacter baumannii* samples. The mean diameter of the inhibition zone for different extracts were; 9.967 ± 6.139 mm at the concentration of 4000 ppm, at the concentration of 400 ppm 13.37 ± 5.45 mm, 13.53 ± 5.49 mm at the concentration of 200 ppm, 14.77 ± 5.17 mm at the concentration of 100 ppm and 14.13 ± 5.7806 mm at the concentration of 50 ppm. The mean diameter of inhibition zone of the extract at the concentration of 50 ppm in comparison to the mean diameter of imipenem at the concentration of 50 ppm was statistically significant (p value= 0.027)

(Table 1, 2 and Diagram 1). The mean diameter of inhibition zone of the extract at the concentration of 50 ppm in comparison with the mean diameter of ciprofloxacin at the concentration of 250 ppm was statistically significant (p value=0.027) (diagram 2). The mean diameter of inhibition zone of the extract at the concentration of 50 ppm in comparison with the mean diameter of the colistin at the concentration of 10 µg/disc was statistically significant (p value=0.027) (diagram 3).

Discussion

Echium amoenum, is an Iranian herb which grows in the north of Iran. All existed data only showed different properties of *Echium amoenum* like as a

Table 1: Diameter of the inhibition zone of the methanolic extract of *Echium amoenum* on isolates of *Acinetobacter baumannii*.

Number of bacteria's strain	Concentration of the extract in ppm				
	4000	400	200	100	50
Diameter of the inhibition zone (Standard deviation \pm mean)					
1	12 \pm 1	15 \pm 1	14 \pm 2	15 \pm 2	14 \pm 2
2	14 \pm 1	16 \pm 2	16 \pm 2	16 \pm 1	17 \pm 1
3	0 \pm 0	16 \pm 3	16 \pm 2	17 \pm 1	17 \pm 1
4	14 \pm 2	17 \pm 1	17 \pm 2	18 \pm 1	18 \pm 1
5	0 \pm 0	14 \pm 1	17 \pm 2	18 \pm 1	17 \pm 1
6	14 \pm 2	15 \pm 1	16 \pm 1	16 \pm 1	15 \pm 1
7	14 \pm 1	16 \pm 3	17 \pm 1	18 \pm 3	14 \pm 3
8	13 \pm 3	15 \pm 4	16 \pm 3	18 \pm 2	17 \pm 2
9	0 \pm 0	16 \pm 2	15 \pm 0	17 \pm 2	17 \pm 2
10	15 \pm 2	16 \pm 1	14 \pm 1	14 \pm 2	14 \pm 2
11	14 \pm 2	14 \pm 2	15 \pm 2	17 \pm 3	17 \pm 3
12	13 \pm 2	15 \pm 2	15 \pm 4	16 \pm 3	16 \pm 3
13	14 \pm 2	14 \pm 2	16 \pm 2	16 \pm 1	17 \pm 1
14	14 \pm 1	16 \pm 2	14 \pm 1	16 \pm 1	16 \pm 1
15	13 \pm 1	15 \pm 2	16 \pm 1	13 \pm 1	14 \pm 1
16	14 \pm 1	12 \pm 3	14 \pm 0	16 \pm 1	17 \pm 2
17	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
18	13 \pm 4	15 \pm 2	14 \pm 2	16 \pm 4	16 \pm 2
19	13 \pm 2	14 \pm 1	16 \pm 3	16 \pm 2	16 \pm 2
20	13 \pm 2	16 \pm 1	14 \pm 1	15 \pm 1	14 \pm 2
21	14 \pm 1	16 \pm 5	16 \pm 1	17 \pm 2	16 \pm 1
22	13 \pm 2	16 \pm 1	16 \pm 1	17 \pm 3	17 \pm 1
23	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
24	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
25	14 \pm 3	16 \pm 4	16 \pm 3	17 \pm 0	17 \pm 1
26	14 \pm 2	15 \pm 2	16 \pm 2	17 \pm 2	17 \pm 1
27	13 \pm 1	16 \pm 2	16 \pm 2	17 \pm 1	18 \pm 4
28	14 \pm 1	17 \pm 1	16 \pm 2	17 \pm 1	17 \pm 2
29	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0	0 \pm 0
30	0 \pm 0	18 \pm 2	18 \pm 1	19 \pm 2	18 \pm 2

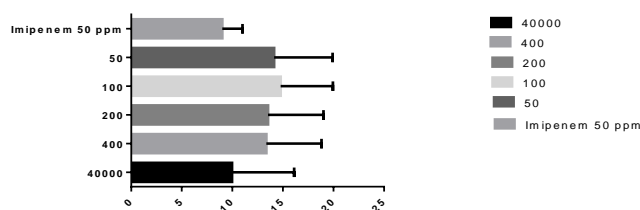


Diagram 1. Diameter of inhibition zone of the methanolic extract of Borage in comparison with Imipenem.

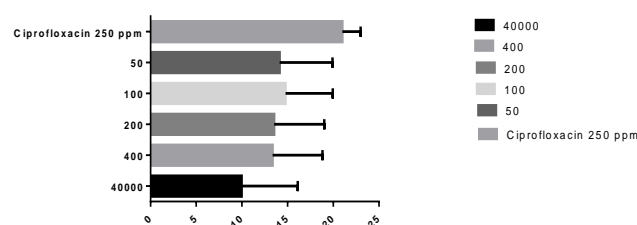


Diagram 2. Diameter of inhibition zone of the methanolic extract of Borage in comparison with ciprofloxacin.

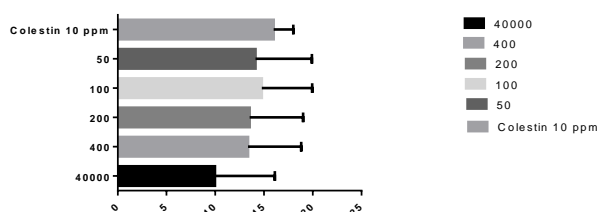


Diagram 3. The diameter of the inhibition zone in mm of the methanolic extract of Borage in comparison with colistin.

mood enhancer, an anxiolytic, anti-inflammatory, anti-laxative, an emollient and also its use such as antiviral against bacteriophage¹³ or as anti *S. aureus* property¹⁵.

In the other hand, infections with *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are two particular concern for hospitalized patients, especially whom with multi drug resistance (MDR) infection¹⁷. Multidrug resistant *Pseudomonas*

aeruginosa and *Acinetobacter baumannii* isolates are increasingly causing hospital acquired infections especially in burned patients.

In this study the antibacterial effect of methanolic extract of *Echium amoenum* (borage) was evaluated against *A. baumannii* isolates from the wound infection of burn patients who referred to Motahari hospital of Tehran for the first time. Based on the results of this study, it was concluded that the best inhibitory concentration for the methanolic extract of borage against *Acinetobacter baumannii* isolates was at the concentration of 100 ppm. Also, the most useful antibiotics in the common treatment of *A. baumannii* strains are included of imipenem, ciprofloxacin and colistin which are recommended by Clinical and Laboratory Standards Institute (CLSI) to evaluate during disc diffusion test¹⁶. The results showed that the clinical strains of the *A. baumannii* were almost highly resistant to imipenem which is the common choice of antibiotic therapy in the hospitals and it may

Table 2: The evaluation of the methanolic extract of *Echium amoenum* on isolated *Acinetobacter baumannii* strains.

	The concentration of the extract in ppm				
	4000	400	200	100	50
MINIMUM	0.0	0.0	0.0	0.0	0.0
MAXIMUM	15.0	18.0	18.0	19.0	19.0
MIDDLE	13.0	15.0	16.0	16.0	16.5
AVERAGE	9.97	13.37	13.53	14.77	14.13
STANDARD DEVIATION	6.14	5.45	5.49	5.17	5.78
NUMBER	30	30	30	30	30

be resulted of the inappropriate prescription of imipenem, now days. So, due to the calculated p value ≤ 0.05 in this study, it can say that borage extract can be as good as or even better than the imipenem which is used in the hospitals now.

By the comparison of the ciprofloxacin effectiveness to methanolic extract we could say that the strains were sensitive to high concentration of ciprofloxacin (250 ppm) in comparison to the borage methanolic extract at the lower concentration (50 ppm), the statistic p value also confirm the accuracy of our result too (p value= 0.027).

Also, the best sensitivity among *A.baumannii* isolates were evaluated in this study was detected to colistin which is almost the last line of antibiotic therapy due to its side effects. So, based on the effectiveness of methanolic extract at the concentration of 50 ppm in comparison to colistin, we concluded that the methanolic extract of borage which is a local herb of Iran and had a strong history in Iranian folk could be more effective against MDR isolates of *A. baumannii* in comparison to the common antibiotics.

Conclusion

Fractionations of the methanolic extract of borage to find the more effective part of it and evaluate its antibacterial effect in the further studies are recommended. Also, doing other *in vitro* tests such as cytotoxicity and mutagenicity and *in vivo* studies of methanolic extract in the future is needed to substitute borage methanolic extract to common antibiotics as an alternative treatment.

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