Antibacterial Effect of Borage (*Echium amoenum*) on *Staphylococcus aureus*

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Borage (*Echium amoenum*) is a large annual plant of the Boraginaceae family, which grows in most of Europe and in northern Iran. The borage flower is used as a medicinal herb in France and other countries. Iranian borage is used in traditional medicine for infectious diseases, flu and as an anti-febrile. We tested the aqueous extract of borage dried flowers in vitro for its antibacterial activity. The extract showed concentration-dependent antibacterial activity against *Staphylococcus aureus* 8327. This activity was heat resistant, but the activity of freeze-dried extract gradually diminished during a 90-day period. The traditional use of Iranian borage flowers for infectious diseases and for controlling fever appears to be justified.

Key Words: Borago, *Echium amoenum*, antibacterial activity, *Staphylococcus aureus*.

Borage (*Echium amaenum*) is a large hairy annual herb that is a member of Boraginaceae family [1]. It grows in most of Europe, in the Mediterranean region, and also in northern parts of Iran. The flowers are bright blue and star-shaped and the fruit consists of four brownish-black nutlets. Borage flourishes in 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 sown in good light soil from mid of March to May [2].

The flowers and the leaves of borage are used medicinally in France as an antifebrile, anti-depressive, for the treatment of stress and of circulatory heart diseases, for pulmonary complaints, as a poultice for inflammatory swellings [3,4], as a diuretic (due to potassium nitrate), as a laxative, emollient and demulcent (due to the mucilage), and recently as a possible protective factor against cancer [5]. The plant constituents have been isolated by different investigators; they include gamma-linolenic acid (GLA), alpha-linolenic acid (ALA), delta6-fatty acyl desaturase, delta8-sphingolipid desaturase [6], pyrrolizidine alkaloids, mucilage, resin, potassium nitrate, and calcium salt combined with mineral acids.

We tested an aqueous extract of dried borage flowers in vitro for its antibacterial activity against *Staphylococcus aureus* 8327.

Materials and Methods

Plant and extract

The borage found in Iran is *Echium amoenum* (F.M.), which is different from the borage grown in Europe, *Borago officialis* L. (*Boraginaceae*). Dried borage flowers were collected from Ardebil province, in northern Iran in mid August. Cold aqueous extract (pH 5.8) of dried *E. amaenum* flowers (5%, w/v) was used in all the experiments. Dried flowers (15 g) were steeped for 6 h at 4°C in 300 mL distilled water, with constant stirring. The material was centrifuged and the supernatant was filter-sterilized and then freeze-dried.

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Anti-bacterial effect of extract

Staphylococcus aureus 8327 was obtained from Tehran University of Medical Sciences, Faculty of Health, Iran. Nutrient broth (NB) containing 5 g peptone (Difco), 5 g NaCl and 3 g beef extract in 1 liter of distilled water (pH 7.5) was used as a culture medium. Anti-bacterial activity of the extract was determined by agar-well diffusion, disc diffusion [7], and the minimum inhibitory concentration (MIC) methods [8]. In the agar-well diffusion method, 9 mm diameter wells were prepared on agar containing 0.5 mL of bacteria ($2x10^{11}$ cells/mL). Freeze-dried extract was diluted 1:20 and different concentrations (1.25 to 10 mg) were added to the wells. In the disc diffusion method, paper discs were soaked in extract solutions and were placed on the bacteria. After 24 h at 37°C, the inhibition zones were measured.

To determine MIC, 5 mL medium was added to six tubes. In the first tube 5 mL extract (1:20 dilution, 50 mg/mL) was added and after mixing 5 mL was removed and added to the second tube; the dilutions continued for all the tubes. Then, 14 mL medium and one mL bacteria suspension were added and the tubes were incubated for 24 and 48 hr at 37°C.

Chromatography

Thin layer chromatography was used to identify the active ingredients of the aqueous extract. Chromatography was performed for 15 h using butanol:acetic acid:distilled water (5:1:4) solvent on a Whatman #1 filter paper. Spots were stained with ninhydrin (to detect amino acids and flavenoids), bismuth iodine, 3% ferric chloride (to detect esters of carboxylic acids and anhydrides), and with Fehling's A+B solution.

Results and Discussion

To determine the antibacterial effect of borage flowers, an aqueous extract was prepared. To get the best aqueous extraction, distilled water with three different pHs, 5.8, 7.0 and 8.5, was used, and about 8.2, 6.8 and 7.0 g lyophilized powder were obtained, respectively, from 15 g dried flowers. The agar-well diffusion method with 1:20 dilution of these different extracts gave inhibition zone diameters of 10, 7 and 6 mm at pH 5.8, 7.0 and 8.5, respectively. Therefore, pH 8.5 was selected for extraction. Table 1 shows antibacterial effects of various concentrations of borage extract with two different methods. The activity was bactericidal, since incubation of the inhibition zone for one week did not show any growth of bacteria.

The inhibitory effect of extract was not due to the pH of the extract, since extract with all three pHs of 5.8, 7.0 and 8.5 had antibacterial activity, and the control pH had no effect. These data indicate that the antiviral activity of the extract is due to the borage component. The MIC of extract on *Staphylococcus aureus* 8327 after 24 and 48 h was determined to be 6.2 mg/mL. Lower dilutions had no anti-bacterial effect.

The anti-bacterial activity of the extract was heat resistant. Autoclaving the extract at 110ºC for one hour did not eliminate its antibacterial activity, and the effect was similar to that of the extract that was filter sterilized. When 200 µL of 1:20 dilution of extract was used in 9 mm diameter wells, in both cases the inhibition zones were 12 mm. The stability assay showed that the anti-bacterial effect of the freeze-dried extract diminished during 90 days storage at 4ºC (Figure 1), and the activity of the working solution was diminished after one week at 4ºC.

These results indicate that the traditional use of the Iranian Borage flower for infectious diseases and for antifebrile activity may be justified. Borage syrup was thought not only to be good for fever, but also to be a remedy for jaundice, itch and ringworm [2]. Also, we have found that borage extract has anti-viral activity (unpublished data). Already, several components, such as linolenic acid, delta6-fatty acyl desaturase, delta 8-sphingolipid desaturase [6], and pyrrolizidine alkaloids, have been isolated and characterized. In the chromatography experiment, when filter paper was stained with different reagents (Table 2), several spots with different colors and different Rf were obtained. These spots show that the aqueous extract has amino

Figure 1. Stability of antibacterial activity of Borago extract during 90 days by agar-well plate. Freeze-dried extract was prepared (5% W/V) and 0.2μm was added to the wells.

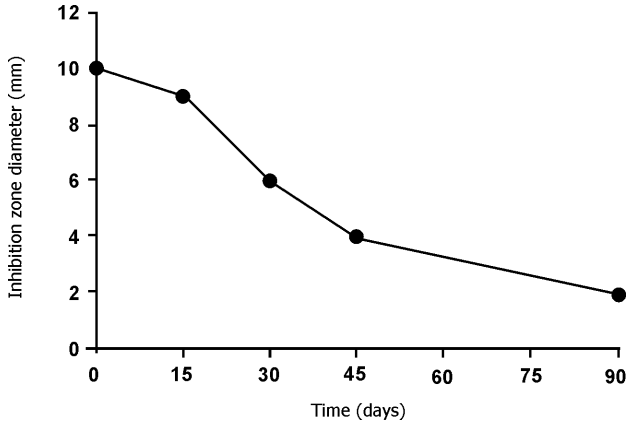

Table 1. Antibacterial effect of Borage flower extract on *Staphylococcus aureus*

Method	Extract (mg)	Inhibition zone diameter (mm)
Disc diffusion	4	8
	1	4
	0.5	0
	0.1	0
Agar-well diffusion	10	10
	5	4
	2.5	0
	1.25	0

Table 2. Thin layer chromatography of aqueous Borage extract on Whatman #1 paper using butanol:acetic acid: water

Reagents	Rf
Ninhydrin	0.13, 0.18, 0.23, 0.38, 0.48, 0.63 (all purple)
Ferric chloride (3%)	0.24 (white), 0. 61 (brown)
Fehling (A+B)	0.21 (yellow)
Bismuth iodine	No spot

acids, but no alkaloids. No antibacterial activity was identified when these spots were placed on bacterial culture. It seems that the materials in these spots are not enough for anti-bacterial activity; however, more data are needed to determine the active anti-bacterial components of the extract

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