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ANTIBACTERIAL EFFECT OF *EUPHORBIA PULCHERIMA* ON *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Bente- qunsul (*Euphorbia pulcherima* L.) is an ornamental and medicinal plant in Iran. It belongs to the family Euphorbiaceae, which grows in most of Europe and widely in northern Iran. We tested the aqueous leaf extract of *E. pulcherima* 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 Bente-qunsul for infectious diseases and for controlling fever appears to be justified.

Key Words: *Euphorbia pulcherima*, antibacterial activity, *Staphylococcus aureus*

INTRODUCTION

Medicinal plants contain physiologically active principles that over the years have been exploited in traditional medicine for the treatment of various ailments (Adebanjo *et al*., 1983; Ebadi *et al.*, 2005 ; Shokrzadeh *et al*., 2005) as they contain anti-microbial properties (Sokmen *et al*., 1999; Kelmanson *et al*., 2000; Srinivasan *et al*., 2001). These medicinal herbs constitute indispensable components of the traditional medicine practiced worldwide due to the low cost, easy access and ancestral experience (Martin-Bettolo, 1980). One such plant *E. pulcherima* (Euphorbiaceae) that called Bente- qonsul locally is an ornamental and medicinal herb that widely cultivated in most parks and gardens in Iran.

The plant has been reported to contain caudicifolin, methylellagic acid (Rastogi and Mehrotra, 1995) and Euphol (Pullaiah, 2002). The paste of rootstock along with mustard oil has the potential for the treatment of rheumatism, gout, arthritis and paralysis (Prakash and Singh, 2001). Other uses of Euphorbia fusiformis includes the roots and leaves for treating fever (Asolkar *et al*., 1992) and the whole plant is used as antiarthiritic and anti-inflammatory (Singh *et al*., 1984; Pullaiah, 2002).

However, there is no report regarding the anti-microbial activity of this plant. Therefore, the aim of the present work was to evaluate the anti-bacterial potentiality of the leaf extract of *E. pulcherima* against the growth of several human pathogenic bacterial strains. We tested an aqueous extract of dried leaf of *E. pulcherima* in vitro for its antibacterial activity against *Staphylococcus aureus* 8327.

MATERIALS AND METHODS

Plant and extract

Plant samples were collected from Mazandaran province, in northern of Iran in mid August (2005). Cold aqueous extract (pH 5.8) of dried *E. pulcherima* (5%, w/v) was used in all the experiments. Dried leaf Sample (15 g) was 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.

Anti-bacterial effect of extract

Staphylococcus aureus 8327 was obtained from Pasteur institute of Tehran, 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 based on Palambo and Semple (2001) and the minimum inhibitory concentration (MIC) methods (Alasbahi *et al*., 1999). 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 (Ahmad *et al*., 1998).

RESULTS

To determine the antibacterial effect of *E. pulcherima*, an aqueous extract of dried leaf 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 *E. pulcherima* 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 *E.pulcherima* component. The MIC of extract on *S. 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 mL 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 antibacterial effect of the freeze-dried extract diminished during 90 days storage at 4ºC (Table 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 *E. pulcherima* for infectious diseases and for antifebrile activity may be justified.

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 acids (Table 3). 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 (Rabe and Staden, 1997; Rastogi and Mehrotra, 1995; Rosoanaivo and Ratsimanaga-Urverg, 1993; Sinclair and Dhingra, 1995).

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

Time (days)	Inhibition zone diameter (mm)
0	10.2
15	8.5
30	6.4
45	4.2
60	3.3
75	3.2
90	2.3

There are several reports stating that other *Euphorbia* species extracts exhibit anti-bacterial activity. Evans et al. (2002) have assayed anti-bacterial activity of Euphorbia hirta, against the typhoid causing organism mainly *Salmonella typhii*. According to another report the ethanolic extract of *E. australis* showed activity against *Bacillus cereus, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa* and *S. typhimurium* (Palambo and Semple, 2001). Similarly the ethanol, acetone and water extracts of *E. fruticosa* (Alasbahi *et al*., 1999) and methanol extracts of *E. macroclada* (Darwish *et al*., 2002) showed some inhibitory effects against selected bacterial strains like *S. aureus.*

Table 2. Antibacterial effect of *E.pulcherima* extract on *Staphylococcus aureus*

Method	Extract (mg)	Inhibition zone diameter (mm)
Disc diffusion	4	8.1
	1	4.1
	0.5	0
	0.1	0
Agar-well diffusion	10	10.3
	5	4.3
	2.5	0
	1.25	0

Table 3. Thin layer chromatography of aqueous *E.pulcherima* extract on Whatman #1 paper using butanol:acetic acid:water

Reagents	Rf
Ninhydrin	0.14, 0.17, 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

This is the pioneering study to demonstrate anti-bacterial activity of *E. fusiformis* using different extracts. Among them, the methanol extract was found to be more active followed by acetone and other extracts. These results confirmed the results of earlier studies that methanol is a better solvent for more consistent extraction of anti-microbial substances from medicinal plants compared to other extracts (Ahmad *et al*., 1998; Eloff, 1998; Lin *et al*., 1999; Karaman *et al*., 2003; Omer and Elnima, 2003). In fine, the rootstock extract of the plant had potential anti-bacterial properties than leaf extracts. The overall performance of this result, the well-in agar method is best suited for studying anti-bacterial activity than disc diffusion method. These results also support the popular use of these plants in tribal/traditional medicine for the treatment of fever, wound infections, and intestinal disorders. Although, the tested plant extracts may contain anti-microbial constituents, further phytochemical and pharmacological studies will be necessary to isolate the active constituents and evaluate the anti-bacterial activity against a wide range of microbial populations (Boaky-Yiadon, 1979; Britto *et al*., 2002; Iennette, 1985; Natarajan *et al*., 2004).

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