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Antibacterial effects of various extracts of *Amygdalus eburnea* on some most common bacteria in burning

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ABSTRACT

Amygdalus eburnea Spach. (Rosaceae family) is a perennial herb that has been valued for its important biological perspectives and it has been used to treat the burn. In this study, antimicrobial activity of *A. eburnea* extract was investigated against some most common bacteria in burning. Antimicrobial effects of *A. eburnea* was performed using Minimum Inhibitory Concentration (MIC) methods on *Staphylococcus aureus* (ATCC 2523), *Staphylococcus epidermidis* (ATCC 1435), and *Pseudomonas aeruginosa* (ATCC 25923). Results demonstrated that both aqueous and menthanolic extract of *A. eburnea* at all concentration had antibacterial activity against the tested microorganisms from week to potent with the MIC ranging from 1.33 to 9.33 mg/mL and 3.33 to 16 mg/mL, respectively. To conclude, the present study showed that the extract from the *A. eburnea* were found to be more active against Gram-positive bacteria as an available and inexpensive herb possesses wound healing activity, and thus provided the evidence for its traditional use value and it is suitable substitute in healing of burn wounds.

Keywords: *Amygdalus eburnea*; MIC; *Staphylococcus aureus*; *Staphylococcus epidermidis*; *Pseudomonas aeruginosa*

INTRODUCTION

Nowadays pharmacological industries have produced a wide range of the new antibiotics; however, since last decades, some antibiotics have become less and less effective against certain illnesses not, only because many of them produce toxic reactions, but also due to emergence of drug-resistant bacteria. Therefore, it is essential to investigate newer drugs with lesser resistance [1, 2].

Since last decades, plant extracts and plant-derived compounds, due to having few side effects, low cost, and high availability, have been valuable sources that are commonly used to treat a wide range of disease conditions including infectious diseases [3, 4]. Antimicrobial properties of medicinal plants are being increasingly reported from different parts of the world. The World Health Organization estimates that plant extracts or their active constituents are used as folk medicine in traditional therapies of 80% of the world's population [5, 6].

Amygdalus eburnea Spach. (Rosaceae family) is a type of almond which is naturally grown and distributed in Iran. This plant is called "Ghosk" in Persian, [7]. In traditional Iranian medicine *A. eburnea* has also been used as laxative and anti-worm. Moreover, brew of dermal tissue are used for cough, respiratory distress and paregoric [8].

The present study aims to evaluate wound healing activity of *A. eburnea* extract and its antifungal and antibacterial effects are also investigated and compared with silver sulfadiazine (a synthetic burn ointment) for treating the induced third degree burn in rat. The present study aims to evaluate the antibacterial effects of merthanolic and

aqueous extract of *A. eburnea* against some most common bacteria in burning such as *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Pseudomonas aeruginosa* to detect new sources of antimicrobial agents.

MATERIALS AND METHODS

Collection of plant materials

The shell root of *A. eburnean* was collected from rural regions of from Baft district, south east of Iran, in April 2013. They were identified by a botanist of the Botany Department of Shahid Bahonar University, Kerman, Iran. A voucher specimen of the plant materials was deposited at the Herbarium of Department of Pharmacognosy of School of Pharmacy, Kerman University of Medical Science, Iran (KF 1136) [9].

Preparing of extracts

One hundred gram of powdered plant material was separately extracted by percolation method with methanol (80%) and water successively for 72 h. in room temperature. The extracts were passed through filter paper (Whatman No.3, Sigma, Germany) to remove plant debris. The extracts were finally concentrated in vacuum at 50°C using a rotary evaporator (Heidolph, Germany) and stored at -20°C, until testing [10-12].

Antibacterial Activity

Microorganisms

Bacterial pathogens including *Staphylococcus aureus* (ATCC 2523), *Staphylococcus epidermidis* (ATCC 1435), and *Pseudomonas aeruginosa* (ATCC 25923) were used for the experiment [13, 14].

Cultivation of Bacteria

The bacterial strains were grown in 250-mL Erlenmeyer flasks containing 50 mL MH (Muller Hinton) broth at 37°C on an orbital incubator shaker. The culture flasks were inoculated to 0.1 OD600 with freshly prepared cells grown in MH medium under the same culture conditions. The midlog phase cultures were used for the antibacterial study.

Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration of the compound was performed according to the reference method described elsewhere [15, 16]. The extract was dissolved in water together with 1% dimethyl sulfoxide (DMSO). The initial test concentration (0.5 mg/mL) was serially diluted twofold to obtain the extracts at the concentrations 0.5-16 mg/mL. Each well was inoculated with 5 µL of suspension containing 10⁸ CFU/mL of bacteria for 24 h at 37°C, Silver sulfadiazine as positive control and DMSO as negative control were used. Five µL of tested broth was placed on the sterile MHA plates and sealed in plastic bags to avoid contamination in the laboratory and at respective temperature. The MIC for bacteria was determined as the lowest concentration of the compound inhibiting the visual growth of the test cultures on the agar plate. The experiment was conducted in triplicate.

Table 1. Mean of minimum inhibitory concentration (MIC) of *Amygdalus eburnea* extracts against some pathogenic bacterial strains.

Tested sample (mg/ml)	Bacterial strain		
	<i>Staphylococcus aureus</i>	<i>Staphylococcus epidermidis</i>	<i>Pseudomonas aeruginosa</i>
Methanolic extract	1.33	6.66	9.33
Aqueous extract	3.33	9.33	16
Silver sulfadiazine	6.66	16	16

RESULTS AND DISCUSSION

The results of *in vitro* antifungal assay (MIC) are presented in Table 1. Both aqueous and menthanolic extract of *A. eburnea* at all concentration had antibacterial activity against the tested microorganisms from week to potent with the MIC ranging from 1.33 to 9.33 mg/mL and 3.33 to 16 mg/mL, respectively. Methanolic extracts significantly ($p < 0.05$) were much more effective than aqueous extract of *A. eburnea* once it exhibited lower MIC values for all the bacteria. Among the tested bacterial pathogens, *S. epidermidis* was the most sensitive to the extracts of *A. eburnean*. Moreover, silver sulfadiazine as control drugs exhibited antibacterial activities with the MIC ranging from 2.66 to 16 mg/ml tested bacterial pathogens.

With the advent of industrial and synthetic antimicrobial agents in the middle of last century, lack of interest in plants as a natural and valuable source for antimicrobial drugs was caused [1]. Recently, with the emergence of some limitations in the use of these drugs, the situation has shifted and field of ethnobotanical research has been expanded [17]. The present study showed that *A. eburnean* extract was capable of inhibiting the growth of microorganisms that are involved in causing burn wound infections. The findings revealed that *A. eburnean* extracts possess activity against gram positive bacteria such as *S. aureus*. Accordingly, the structural differences between the

cell walls of gram-positive and gram-negative bacteria are thought to be responsible [18]. Extract had inhibitory effect on peptidoglycan synthesis so, without peptidoglycan or when the integrity of peptidoglycan is compromised, the bacteria undergo immediate osmotic lysis [19, 20]. But in gram negative bacteria outer membrane act as barrier. However, its antimicrobial activity though their exact mode of action is poorly understood. Further studies are required to elucidate the antimicrobial mechanisms of *A. eburnean* extract. To conclude, the present study showed that the extract from the *A. eburnean* were found to be more active against Gram-positive bacteria as an available and inexpensive herb possesses wound healing activity, and thus provided the evidence for its traditional use value and it is suitable substitute in healing of burn wounds.

Declaration of Interest

The author declares that there is no conflict of interest in this study.

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