IN VITRO INHIBITORY EFFECT OF ALCOHOLIC AND AQUEOUS EXTRACT OF UMBILICUS INTERMEDIUS BOISS (NAFE VENUS) ON STAPHYLOCOCCUS AUREUS AND PSEUDOMONAS AERUGINOSA

Mohamad Ali Roozegar\textsuperscript{1}, Zeynab Pournazari\textsuperscript{2}, Mohamad Reza Nazari\textsuperscript{3}, Mohamad Reza Havasian\textsuperscript{4}, Jafar Panahi\textsuperscript{5}, Iraj Pakzad\textsuperscript{6}

HOW TO CITE THIS ARTICLE:

ABSTRACT: INTRODUCTION: Recently using of plant drug against bacterial infectious for reason drug resistant has special importance. Umbilicus intermedius boiss (nafe venus) in traditional used for treatment of infectious disease in western of Iran. This study aims to evaluated of inhibitory effect of alcoholic and aqueous extract of Umbilicus intermedius boiss (nafe venus) on Staphylococcus aureus and Pseudomonas aeruginosa in vitro. METHODS: The plant was collected and dried in worm and low humid environment alcoholic and aqueous extract prepared according to the standard method. Clinical samples of Staphylococcus aureus and Pseudomonas aeruginosa was used. Inhibitory effect of alcoholic and aqueous extract was done in Muller Hinton Agar medium by disk diffusion method. RESULTS: Inhibitory zone of alcoholic extract on S. aureus and P. aeruginosa was 14mm and 10mm respectively. Zone of inhibition of aqueous extract for S. aureus and P. aeruginosa was 20mm and 14mm respectively. MIC of aqueous extract for S. aureus and P. aeruginosa was 10 and 15ug/ml and MIC of alcoholic extract was 15 and 20ug/ml respectively. MBC of aqueous extract for S. aureus and P. aeruginosa 15 and 25ug/ml and MBC of alcoholic extract were 25 and 30ug/ml respectively. CONCLUSION: The aqueous extract had higher antibacterial effect than the alcoholic extract. This extract had higher effect on gram negative bacteria than gram positive. KEYWORDS: Staphylococcus aureus, Pseudomonas aeruginosa, MIC, Disk diffusion method.

INTRODUCTION: In recent years, increasing of resistant strains of bacteria results some antibiotics have lost their effectiveness.\textsuperscript{(1)} Indeed, the view returns to years ago that some plants are treatable\textsuperscript{(2)} Umbilicus intermedius boiss (nafe venus) is from crassulaceae family and thriving in the desert, and has a greenish-pink flowers, and the plants used in traditional medicine, especially in southern regions of west Iran Ilam, which is traditionally used to treat various infections. Indeed, the emergence of antibiotic-resistant strains of Staphylococcus aureus and the presence of intrinsic resistance in Pseudomonas aeruginosa strains is causing more attention to the antibacterial properties of medicinal plants.\textsuperscript{(3-6)}

Pseudomonas aeruginosa is a Gram-negative pathogenic bacterium that including of diseases factors in patients with a weakened immune system, which can be lead to severe infections and death.\textsuperscript{(7,8)} Antibacterial and antifungal effects of some plant extracts have been shown in several studies\textsuperscript{(9,10)} traditionally, Umbilicus intermedius boiss has been used to treat infections in the West region of Iran, thus this study aim to evaluate of inhibitory effect of alcoholic and aqueous extract of Umbilicus intermedius boiss(nafe venus on Staphylococcus aureus and Pseudomonas aeruginosa in vitro.
METHODS: Sample Collection: 30 Clinical samples of Pseudomonas aeruginosa and Staphylococcus aureus were collected from hospitals in city of Ilam. The samples were confirmed by using standard methods of diagnosis and were cultured in TSB medium and were kept at minus 20 degrees celsius. (11)

Alcoholic Extract: The plant was collected from the desert south of Ilam and washed and dried away from direct sunlight in the shade and then 10gms of plant powder mixed in 250 ml of 80% ethanol solution for 48 hours at room temperature on surface of the horizon was shaken (150 rpm). The extract cleared two times by filter paper and the alcohol was evaporated by using a rotary machine. Then 10% dissolved of extract prepared in 80% ethanol and stored in dark glass container at 4°C. (12)

Aqueous Extract: 10 grams of the herb powder was mixed with 200 ml of boiling distilled water and was put on the heater stride device for 20 minutes along with mixing. Then the mixture was filtered using a fine fibred sterile cloth and was centrifuged at 3500 rpm for 15 minutes. The mixture was left in open air until the solvent have completely evaporated. (9)

Disk Diffusion Method: First a suspension of the bacteria being studied was prepared with a concentration of 0.5 McFarland and using the a sterile valve on top of the plates containing Mueller Hinton Agar culture media, the bacteria were cultivated. After the surface of the plate had dried, paper disks with a diameter of 6mm, impregnated with 10μl of the extract with different concentrations of 10, 20, 40 and 80 mg/ml were placed on the plates and they were incubated for 24 hours at 37°C. After incubation the inhibition zone was measure using a ruler. (13)

Determining MIC: Different concentrations of aqueous extract were added to similar volumes of bacteria suspension equal to 10^5 CFU/ml of staphylococcus aureus and pseudomonas aeruginosa in BHI liquid culture medium and after 24 hours incubation at 37°C and the MIC was determined according to SLCI instructions.

Determining MBC: To determine MBC, 100 μl of three of the concentrations prior to MIC were cultivated separately on Mueller Hinton Agar and the concentration in which no bacteria had grown would be the MBC. (14)

RESULTS:

Results of the inhibitory effect of aqueous and alcoholic extract via the disk diffusion method:

In this method, the minimum and maximum inhibitory effects of the aqueous extract on staphylococcus aureus were at 10 and 80mg/ml consecutively and the inhibition zones for these concentrations were 16.5 and 23.2 mm consecutively. The minimum and maximum inhibitory effects of the extract on pseudomonas aeruginosa were at 10 and 80mg/ml consecutively and the inhibition zones for these concentrations were 10 and 18 mm consecutively (table 1).

Minimum and maximum inhibitory effects of the alcoholic extract on staphylococcus aureus were at 10 and 80mg/ml consecutively and the inhibition zones for these concentrations were 8 and 19 mm consecutively. The minimum and maximum inhibitory effects of the extract on pseudomonas aeruginosa were at 10 and 80mg/ml consecutively and the inhibition zones for these concentrations were 8.4 and 12.2 mm consecutively (table 1).
MIC and MBC results of the aqueous and alcoholic extract on staphylococcus aureus and pseudomonas aeruginosa: The results obtained for determining the MIC and MBC of the extract on staphylococcus aureus were 10μg/ml and 15μg/ml consecutively. The MIC and MBC of the extract on pseudomonas aeruginosa were 15μg/ml and 25μg/ml consecutively (table 2). The results obtained for determining the MIC and MBC of the extract on staphylococcus aureus were 15μg/ml and 25μg/ml consecutively. The MIC and MBC of the extract on pseudomonas aeruginosa were 15μg/ml and 30μg/ml consecutively (table 2).

DISCUSSION: Staphylococcus aureus is one of the main infectious agents in hospitals that every person catches it one time on her life.15 Also this bacterium is one of the main infectious agents in patient with weakness immune system.16 Since conventional antibiotics have side effects, drug resistance, and high economic costs on society and family, While their popularity provided use of herbs.17

Today, much attention has been using herbs. In this study, was studied the inhibitory effect of aqueous and alcoholic extracts of Umbilicus intermedius boiss against Staphylococcus aureus and Pseudomonas aeruginosa on in vitro. According to the traditional use of the plant for treatment of infections, expected to the extracts have strong antimicrobial effects.

As the results became clear aqueous extract of Umbilicus intermedius boiss had stronger antimicrobial activity than the alcoholic extract against Staphylococcus aureus and Pseudomonas aeruginosa. Also in Behdani et al.’s study it was shown that Henna extract also has a stronger inhibitory effect compared to the study at hand on staphylococcus aureus and pseudomonas aeruginosa.2

In another similar study to verify the antibacterial activity of henna against Staphylococcus aureus and Pseudomonas aeruginosa was already mostly obtained results18,19 that indicated the low inhibitory effect of those plant compared with this. Dadgar et al at 2007 by experiment of 20 different plant against bacteria indicated that Anaria has most effect against bacteria.20

Haydary et al study in 2013 to evaluate the inhibitory effect of aqueous and alcoholic extracts of Satureja bachtiarica on Escherichia coli and Staphylococcus aureus indicated that MIC of alcoholic and aqueous extract was 8, 32μg/ml and MBC was 16 and 64 respectively. That against this study, alcoholic extract has more effect compared with this plant.21

Ahmadi et al study about evaluated inhibitory effect of alcoholic and aqueous extract of Azgil against bacteria indicated that those extract has low effect against these bacteria compared with Umbilicus intermedius.22 Alizade et al indicated Lavandula stoechas has more effective against gram positive bacteria compared with gram negative bacteria.23

CONCLUSION: According to the obtained result, alcoholic extract of Umbilicus intermedius boiss has significant inhibitory effect on acinetobacter and also inhibitory effect of this extract against positive gram bacteria was more than negative gram bacteria.

REFERENCES:


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<table>
<thead>
<tr>
<th>Samples</th>
<th>Concentration mg/ml</th>
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<tr>
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Table 1: Results of disk diffusion method
### Table 2: Results of MIC and MBC

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**AUTHORS:**
1. Mohamad Ali Roozegar
2. Zeynab Pournazari
3. Mohamad Reza Nazari
4. Mohamad Reza Havasian
5. Jafar Panahi
6. Iraj Pakzad

**PARTICULARS OF CONTRIBUTORS:**
1. Assistant Professor, Department of Periodontology, Faculty of Dentistry, Ilam University of Medical Sciences, Ilam, Iran.
2. Student, Department of Chemistry, Islamic Azad University, Branch of Ilam.
3. Student, Department of Microbiology, Laboratoies Group, Science Research of Azad University of Ilam, Branch of Ilam.
4. Student, Student Research of Committee, Ilam University of Medical Sciences, Ilam, Iran.
5. Assistant Professor, Department of Microbiology, Faculty of Medicine, Ilam University of Medical Sciences, Ilam, Iran.
6. Assistant Professor, Department of Microbiology, Clinical Microbiology Research Center, Ilam University of Medical Sciences, Ilam, Iran.

**NAME ADDRESS EMAIL ID OF THE CORRESPONDING AUTHOR:**
Dr. Iraj Pakzad,
Clinical Microbiology Research Center,
Ilam University of Medical Sciences,
Ilam, Iran.
Email: pakzad_i2006@yahoo.com