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

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ABSTRACT: INTRODUCTION: Recently, using plant drugs against drug resistant bacterial infections is gaining special importance. Umbilicus intermedius boiss (nafe venus) is a traditional remedy used for treatment of infectious disease in western of Iran. This study aims to the evaluate 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 warm and low humidity environment. Alcoholic and aqueous extract prepared according to the standard method. Clinical samples of Staphylococcus aureus and Pseudomonas aeruginosa was used. The inhibitory effect of alcoholic and aqueous extract was done in the 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 15ugr/ml and MIC of alcoholic extract was 15 and 20ugr/ml respectively. MBC of aqueous extract for S. aureus and P. aeruginosa 15 and 25ugr/ml and MBC of alcoholic extract were 25 and 30ugr/ml respectively. **CONCLUSION:** The aqueous extract had a higher antibacterial effect than the alcoholic extract. This extract had a higher effect on gram negative bacteria than a gram positive. **KEYWORDS:** Staphylococcus aureus, Pseudomonas aeruginosa, MIC, Disk diffusion method.

INTRODUCTION: In recent years, due to increasing of resistant strains of bacteria, some antibiotics have lost their effectiveness. ⁽¹⁾ Indeed, the view returns to years ago that some plants are treatable⁽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.⁽³⁻⁶⁾

Pseudomonas aeruginosa is a Gram-negative pathogenic bacterium that including of diseases factors in patients with a weakened immune system, which can lead to severe infections and death.^(7,8) Antibacterial and antifungal effects of some plant extracts have been shown in several studies^(9,10) Traditionally, Umbilicus intermedius boiss has been used to treat infections in the West region of Iran, thus this study aims to evaluate the inhibitory effect of alcoholic and aqueous extract of Umbilicus intermedius boiss(nafe venus on Staphylococcus aureus and Pseudomonas aeruginosa in vitro.

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METHODS: Sample Collection: 30 Clinical samples of *Pseudomonas aeruginosa* and *Staphylococcus aureus* were collected from hospitals in the 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 the 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 containers at 4°C.⁽¹²⁾

Aqueous Extract: 10 grams of the herb powder were 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 measured using a ruler.⁽¹³⁾

Determining MIC: Different concentrations of aqueous extract were added to similar volumes of bacteria suspension equal to 10⁵ CFU/ml of *staphylococcus aureus* and *Pseudomonas aeruginosa* in BHI liquid culture medium and after 24hours 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).

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MIC and MBC results of the aqueous and alcoholic extract on *Staphylococcus aureus* and *Pseudomonas aeruginosa*: The results obtained in 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 in 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 at least..⁽¹⁵⁾ Also, this bacterium is one of the main infectious agents in patient with weakened immune system.⁽¹⁶⁾ Since conventional antibiotics have side effects, drug resistance, and high economic costs to society and family, While their popularity provided use of herbs.⁽¹⁷⁾

Today, much attention has been using herbs. In this study, we 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 results^(18,19) that indicated the low inhibitory effect of those plant compared with this. Dadgar et al at 2007 by experiment of 20 different plants 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 s about evaluating the inhibitory effect of alcoholic and aqueous extract of *Azgil* against bacteria indicated that these extracts have low effective 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 a significant inhibitory effect and also inhibitory effect of this extract against positive gram bacteria was more than negative gram bacteria.

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	Samples	Concentration mg/ml	Zone of growth inhibition
Aqueous extract	pseudomonas aeruginosa	10	10mm
		20	14mm
		40	15mm
		80	18mm
	Staphylococcus Aureus	10	16.5mm
		20	19mm
		40	21.3mm
		80	23.3mm
Alcoholic extract	pseudomonas aeruginosa	10	8.4mm
		20	9.5mm
		40	10.3mm
		80	12.2mm
	Staphylococcus aureus	10	8mm
		20	13mm
		40	16mm
		80	19mm

Table 1: Results of disk diffusion method

	Extracts	Samples	
MIC	Aqueous extract	pseudomonas aeruginosa	15
		Staphylococcus aureus	10
	Alcoholic extract	Pseudomonas aeruginosa	20
		Staphylococcus aureus	15
MBC	Aqueous extract	Pseudomonas aeruginosa	25
		Staphylococcus aureus	15
	Alcoholic extract	Pseudomonas aeruginosa	30
		Staphylococcus aureus	25

Table 2: Results of MIC and MBC

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