

Inhibitory Effects of a Herbal Ointment against *Pseudomonas aeruginosa*

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ABSTRACT

Background and Objectives: *Pseudomonas aeruginosa* is a gram negative opportunistic pathogen and an important cause of wound infections and nosocomial infections. The purpose of this study was to study inhibitory effects of a new ointment prepared from medicinal plants against *P. aeruginosa* isolates.

Methods: In this study, an ointment called ZOUSH was prepared from mixing alcoholic extracts of *Satureja khuzestanica*, *Zataria multiflora*, *Mentha mozaffariani Jamzad*, honey and polyurethane. Minimal inhibitory concentration of ZOUSH and its compositions alone or combined was determined using the disk diffusion method.

Results: *S. khuzestanica*, *Z. multiflora* and *Mentha mozaffariani Jamzad* had inhibitory effects against *P. aeruginosa*. The ZOUSH ointment had greater antibacterial effects than the any of its compositions used solely or combined. The diameter of inhibition zone had a direct relationship with the concentration of the extracts. Moreover, the antibacterial effect of the ZOUSH ointment was identical to that of polymyxine B (300 µg).

Conclusion: We demonstrated that the ZOUSH ointment has inhibitory effects against *P. aeruginosa*. The inhibition zone diameter is directly correlated with the concentration of the extracts. Our results suggest that the ointment could be useful for treatment of burn wounds and skin infections.

Keywords: *Pseudomonas aeruginosa*, ZOUSH, Burns, *Satureja khuzestanica*, *Zataria multiflora*, *Mentha Mozaffariani Jamzad*, Honey, Polyurethane, Ointment.

INTRODUCTION

Pseudomonas aeruginosa is the third most common cause of nosocomial infections and the second most important cause of wound infections (1). Emergence of multi drug resistant (MDR) and extensively drug resistant (EDR) strains of *P. aeruginosa* as well as the increased rate of resistant infections in burn patients highlights the importance of infection control.

P. aeruginosa species have various virulence factors such as pili, exo-enzyme A, exotoxins, etc. (2). Infection caused by this bacterium is believed to be responsible for 75% of all mortalities in burn patients (3, 4). Given the high prevalence of β -lactamase production by *P. aeruginosa* and the high rate of antibiotic resistance, researchers have become more interested in seeking medicinal herbs with antibacterial properties that are safe and without side effects of chemical drugs (5). One of the most important health complications is the infections secondary to burn wounds and war wounds. Dau and et al. reported that 7% of war-related injuries are burn wounds (6). *Satureja khuzestanica* is an herbaceous, aromatic and perennial herb from the family *Lamiaceae*. Thyme and *Satureja* are thought to have antimicrobial activities due to presence of phenolic compounds, thymol and carvacrol (7, 8). *Zataria multiflora* and *Oregano (Origanum vulgare L.)* are also medicinal plants from the family *Lamiaceae* (9). It has been suggested that *S. khuzestanica*, *Z. multiflora* and *Mentha mozaffariani Jamzad* may have antibacterial activity, particularly against *P. aeruginosa* (10). In this study, we aimed to assess the antibacterial activity of *S. khuzestaniae*, *Mentha mozaffariani Jamzad* and *Z. multiflora*, honey and polyurethane against *P. aeruginosa* isolates.

MATERIALS AND METHODS

Arial parts of *S. khuzestaniae*, *Mentha mozaffariani Jamzad* and *Z. multiflora* were collected from Ahvaz (southwestern Iran), Kermanshah (western Iran) and Shiraz (south-central Iran), respectively. The collected plants were fragmented to small pieces (200 g) and dried. Alcoholic extractions of the plants were prepared at Barij Essence Kashan, Iran.

P. aeruginosa PAO1 was used as the reference strain. Susceptibility of the isolates to different antibiotics was assessed by disc diffusion

method according to the Clinical Laboratory Standard Institute guidelines (CLSI 2016). The following antibiotic disks were used in the susceptibility testing: polymyxine B (300 U/IE), tobramycin (10 μ g), gentamycin (10 μ g), imipenem (10 μ g) and meropenem (10 μ g). The antimicrobial activity of the plant extracts, honey and polyurethane was also determined by the disk diffusion method. To prepare the ZOUSH ointment (mixture of the plant extract, honey and polyurethane), one gram of each plant extract was dissolved in 3 ml of dimethyl sulfoxide (DMSO), and one gram of honey and one gram of polyurethane were each dissolved in 3 ml of water. *P. aeruginosa* PAO1 (1.5×10^8 CFU/ml) were spread on Muller-Hinton agar plates. Subsequently, disks containing different concentrations of the ointment were placed on the medium. Later, the plates were incubated at 37 °C for 24 hours. The diameter of inhibition zone was measured in mm. A disk previously immersed in DMSO was used as the negative control. The experiments were repeated three times.

Minimal inhibitory concentration (MIC) was determined by broth macro dilution assay according to the National Committee for Clinical Laboratory Standard guidelines. First, a bacterial stock solution with a turbidity equivalent to 0.5 McFarland turbidity standard (1.5×10^8 CFU/ml) was prepared. The plate was incubated at 37 °C for 24 hours. A tube without the ZOUSH stock was used as negative control. A medium without turbidity was considered as resistant. Lowest concentration at which the growth of bacteria was inhibited was determined as the MIC. *P. aeruginosa* was identified by morphological assessment, catalase test, oxidase test, oxidation-reduction test, *Pseudomonas* agar base medium and pigment formation.

RESULTS

All tubes were re-cultured on Muller Hinton agar to determine the MIC values using the broth macrodilution technique. Table 1 shows the results of MIC values for *P. aeruginosa* PAO1 using broth macrodilution and microdilution methods. The results showed that the ZOUSH ointment had greater antibacterial effects than the any of its compositions used solely or combined.

Table 1 -MIC values for the ZOUSH ointment and its compositions

Agent	MIC ($\mu\text{g/mL}$)
<i>S. khuzestanica</i>	0.079
<i>Z. multiflora</i>	0.079
<i>Mentha mozaffariani Jamzad</i>	0.158
Honey	0.079
<i>S. khuzestanica</i> + <i>Z. multiflora</i>	0.059
<i>S. khuzestanica</i> + <i>Mentha mozaffariani Jamzad</i>	0.079
<i>Z. multiflora</i> + <i>Mentha mozaffariani Jamzad</i>	0.079
ZOUSH ointment	0.039

Susceptibility of *P. aeruginosa* PAO1 to the antibiotics was assessed using the Kirby-Bauer method. The results showed that *P. aeruginosa* PAO1 was sensitive to gentamicin, imipenem, meropenem, polymyxine B and tobramycin (Table 2).

The susceptibility of *P. aeruginosa* PAO1 to different concentrations of the ZOUSH and its compositions alone or combined was evaluated. Based on the results, the growth inhibition zone for 20 μg of single extracts (*S. khuzestanica*, *Z. multiflora*, *Mentha*

mozaffariani Jamzad), double extracts (*Z. multiflora* and *S. khuzestanica*, *Mentha mozaffariani Jamzad* and *S. khuzestanica*, *Mentha mozaffariani Jamzad* and *Z. multiflora*), triple extract (*Mentha mozaffariani Jamzad*, *S. khuzestanica* and *Z. multiflora*) and the ZOUSH ointment was almost identical to that of the polymyxin B (300 μg).

In addition, the inhibition zone diameter had a direct relationship with the extract's concentration (Table 3).

Table 3- Diameter of inhibition zone for different concentrations of the ZOUSH ointment and the extracts alone and combined

Agent	10 μg	13 μg	17 μg	20 μg
<i>S. khuzestanica</i>	9 mm	10 mm	12 mm	16 mm
<i>Z. multiflora</i>	8 mm	9 mm	12 mm	14 mm
<i>Mentha mozaffariani Jamzad</i>	9 mm	11 mm	12 mm	15 mm
<i>S. khuzestanica</i> + <i>Z. multiflora</i>	10 mm	12 mm	14 mm	15 mm
<i>S. khuzestanica</i> + <i>Mentha mozaffariani Jamzad</i>	12 mm	14 mm	15 mm	16 mm
<i>Z. multiflora</i> + <i>Mentha mozaffariani Jamzad</i>	10 mm	13 mm	14 mm	16 mm
<i>S. khuzestanica</i> + <i>Z. multiflora</i> + <i>Mentha mozaffariani Jamzad</i>	11 mm	13 mm	14 mm	17 mm
ZOUSH ointment	12 mm	14 mm	15 mm	18 mm

DISCUSSION

P. aeruginosa is an important opportunistic, gram-negative bacillus and a main cause of mortality among immunocompromised patients (11, 12). Burn injuries are one of the most serious medical conditions, which affect the patients both physically and psychologically. Despite the recent scientific and therapeutic advances, burns still remain as a major public health problem around the world, especially in developing countries, such as Iran (13, 14). Moreover, the increasing trend of resistant *P. aeruginosa* infection of burn wounds has raised some concerns, and signified the need

for implementation of effective infection control program for burn patients. However, prevention of *P. aeruginosa* spread in hospital settings is challenging because of the bacterium's intrinsic and acquired resistance to numerous antibiotics. Additionally, the emergence of MDR and XDR strains has become a worldwide health problem. Traditional medicine plays an important role in the treatment and prevention of infections caused by these bacteria (15). Carvacrol is a major component of the *S. khuzestanica* extract, which inhibits ATPase activity and increases bacterial cell membrane

permeability, thus facilitating the access of antibacterial agents (16). In 2015, Farzaneh et al. showed that carvacrol, γ -terpinene and p-cymene are the main components of *S. khuzestanica*. They also demonstrated the antifungal properties of various species of *Satureja* and the antimicrobial activity of ZOUSH ointment compounds (17). Similar to our study, Esmaili et al. reported the inhibitory effect of *S. khuzestanica* against *P. aeruginosa* (18).

In a study conducted by Moghim et al., the antifungal effect of *Z. multiflora* and *Nigella Sativa* was proven against *Candida albicans* (19). Kavooosi et al. investigated the effect of *Z. multiflora* on *Salmonella typhimurium*, *Escherichia coli*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Aspergillus niger* and *C. albicans*. They claimed that the thymol and carvacrol content of *Z. multiflora* has a direct relationship with its antioxidant, antibacterial, antifungal and anti-tumor

properties (20). Our results also confirm the antibacterial properties of *Z. multiflora*.

CONCLUSION

We demonstrated that the ZOUSH ointment has inhibitory effects against *P. aeruginosa*. The inhibition zone diameter is directly correlated with the concentration of the extracts. Our results suggest that the ointment could be useful for treatment of burn wounds and skin infections.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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