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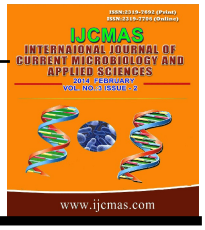

Original Research Article

The Study of Inhibitory Effects of *Satureja khuzestanica* against MDR Isolates of *Pseudomonas aeruginosa*

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A B S T R A C T

Pseudomonas aeruginosa is very highly resistant to chemical antimicrobial agents. These bacteria are one of MDR producing that great potential have for the rapid development of antibiotic resistance. The indecorous use of antibiotics is often associated with adverse effects on the human health. Because of Satureja khuzestanica has antimicrobial properties so they can be used against infections caused by MDR *Pseudomonas aeruginosa*. Satureja khuzestanica has inhibitory properties against Pseudomonas aeruginosa. In this study the antimicrobial effects of Satureja khuzestanica on drug-resistant strains of *Pseudomonas aeruginosa* were investigated using antimicrobial analysis with CLSI 2013 and Dilution and Diffusion method. The agar dilution method results revealed that Satureja khuzestanica had strong inhibitory effects against Multidrug-resistant strains of *Pseudomonas aeruginosa with MIC 80 µg/ml*. Because of proper effects of plant essential oils, with a broader range of studies can be used as a complementary therapy.

Keywords

Inhibitory effects; *Satureja khuzestanica;* MDR; *Pseudomonas aeruginosa*.

Introduction

The prevalence of the inherent drug resistant and acquired relative to antibiotics in *Pseudomonas aeruginosa*, treatment infections *Pseudomonas aeruginosa* caused serious problems in treatment it (Sundheim *et al*., 1998; Amiri *et al*., 2011; Sevillano *et al*., 2006).

For these reasons the use of alternative medicines instead of synthetic antibiotics is rising (Amiri *et al*.,2011). According to many being native medicinal plants in Iran as well as antibacterial effects that they have, the use of these plants are very efficient benefit. In this study the effects

of antibacterial Satureja khuzestanica against Pseudomonas aeruginosa has been examined. Satureja khuzestanica is an endemic plant that is widely distributed in the southern part of Iran. It is famous for its medicine (Ariana *et al*., 2011). Recently, antiviral, antibacterial, antifungal, and antiprotozoal effects were investigated from various species of Satureja khuzestanica (Oussalah *et al.*, 2007). Antimicrobial activity of Satureja khuzestanica, basically is because of main phenolic components, Carvacrol and Thymol, and when they were tested separately, demonstrated wide bacteriocidal activity (Deans and Svoboda,1989). Carvacrol is also found in Thyme, but the distinctive feature of Satureja khuzestanica, is exist of 94% carvacrol in its essence. However the maximum rate is 40% carvacrol in the essential oils of other plants. Carvacrol (2-methyl and 5-isopropyl phenol) can be used as a broad-spectrum antimicrobial activity was introduced. carvacrol has liquid form and a smell similar thymol, and so it has several biological characterize, including the effect of antiseptic, anti-worm, anti-inflammatory activity, analgesic, anti-bacterial, anti-fungal and yeast, antioxidants, and synthesis some organic matter(Deans and Svoboda,1989; Sahin *et al*., 2003).

Carvacrol due to the nature of hydrophobic interactions with the cytoplasmic membrane and cause its deterioration and leakage of cellular materials such as ions, adenosine triphosphate (ATP) and nucleic acid (Dorman and Deans, 2000; Lambert *et al*., 2001). In other words, bactericidal effect of carvacrol is like other phenolic compounds with increased permeability of the bacterial cell membrane to H + and K + ATP depletion and lead to death bacteria (Manohar *et al*., 2001; Nostro *et al*., 2007).

In this study, qualitative and quantitative changes of Satureja Khuzestanica have been studied. Essence extracted was performed with distilled water and Clevenger apparatus was used and identify the compound of essence done with GC-mass. Antibacterial effect of essence identified with the minimum inhibitory concentration method.

Materials and Methods

Antimicrobial effects of *S.khuzestanica*

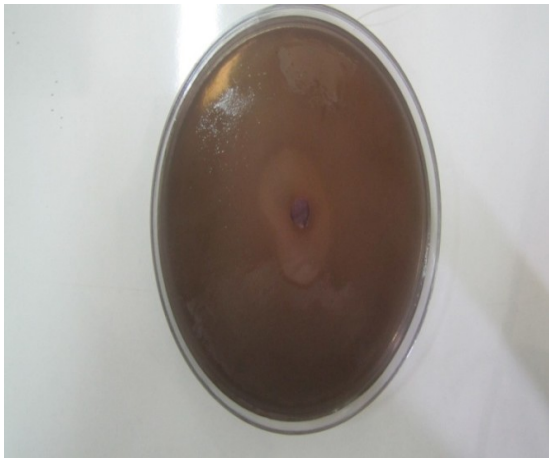
For this study, the microbial strains were collected of Baqiyatallah hospital and the Institute of Scientific and Industrial Research of Iran. For determination of antimicrobial effect of S.*khuzestanica* use of Disc-diffusion and broth dilution method. We prepare a suspension of 24 h cultures of bacteria in Muller Hinton agar with 0.5 MC Farland concentrations. Then 0.5 ml of each bacterial suspension was transferred to Mueller Hinton agar medium with sterile swabs were unevenly distributed. Blank sterile discs containing 10 ml of pure essence were placed on medium. This experiments were done with 2 repeat and Ceftazidime disks were used to compare the antimicrobial effect of essential oils. Plates were incubated at 37 ° C and the inhibition zone was measured after 24 hours. Selected strain was resist to ceftazidim, but in plates containing essence discs the inhibition zone diameter were 25 and 23 millimeter respectively (figure 1).

Determination of Minimum Inhibitory Concentration (MIC)

For supplying microbial suspension, 0.5

MC farland from bacteria to a tube containing 1ml of Hinton broth medium containing 20µl from phenol red 0.4% was added.

Figure.1 Antimicrobial effects of *S.khuzestanica*



For prepare different dilution of essence, at first the essence in a ratio of 1 to 10 with the it's specific solvent, Dimethyl sulfoxide (DMSO), were mixed. DMSO doesn't have any antibacterial properties. Then to tubes containing 1ml of bacterial suspensions were added concentrations 10 to 200 µg of essence. Then the tubes were incubated for 24 hours.

Results and Discussion

Plates review indicated that *Pseudomonas aeruginosa* continue to grow in the volume from 10 to 150 µg /ml of essence, but in the concentration of 160 µg l, showed MIC.So the minimum inhibitory concentration (MIC) for *Satureja khuzestanica* essence is 160 µg/ml.

It is well known that over use of antibiotic caused to increase the rate of resistance isolates. The other hand there are a lot of plant that antimicrobial effect of them is proved. The result indicates that *Satureja khusestanica* has benefit effect on *Pseudomonas aeruginosa*. So we can use of this plant extract instead of synthetic antibiotic or as a complementary medicin. Of course it's necessary for us that knew how *Satureja khuzestanica* cause to eliminate *Pseudomonas aeruginosa* pathogenicity. Therefore we suggest that investigate the effect of *Satureja khusestanica* essence on virulence gene expression in *Pseudomonas aeruginosa.*

Their antimicrobial activity is mainly attributed to the presence of some active constituents in their EOs together with their hydrophobicity which enables them for rupturing cell membranes and intrastructures (Sikkema et al., 1994). In this study, satureja essence were used to assess their antibacterial activity against *important pathogens* by inserting some minor changes to the CLSI recommended agar dilution method that have been originally developed for analyzing the conventional antimicrobial agents activity, so it could be used to analyze plant extracts and essential oils for their antimicrobial activity (Hammer et al.,1999: Van de Braak SAAJ and Leijten,1999; Milhau *et al*., 1997; Al-Shuneigat *et al*., 2005; Horne *et al*., 2001;Calamari *et al*., 2003; NCCLs, 2003;Esmaeili *et al*., 2003). In this study using satureja essence against these pathogens resulted in these which can be effective enough to reduce the rate of infection transmission.

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