



Evaluation of the effect of diluted essential oil of *Zataria multiflora* with nanotechnology on *Pseudomonas aeruginosa* in hospitals

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Abstract

Introduction: *Pseudomonas aeruginosa* is an opportunistic pathogen that has been identified as one of the most important nosocomial pathogens in recent years. This bacterium is responsible for 11 to 13% of nosocomial infections, especially in patients with cystic fibrosis, people with burns or immunodeficiency, and people undergoing artificial ventilation. *Pseudomonas aeruginosa* is highly resistant to many antimicrobials and antiseptics, and the presence of strains with multiple drug resistance is a major problem in the treatment of bacteria in important hospital wards such as burns and intensive care. The aim of this study was to evaluate the effect of thyme essential oil on resistant *Pseudomonas* isolated from patients with cystic fibrosis.

methods: First, the bacterium was isolated from the sputum sample of a cystic fibrosis patient and its identity was determined and its pattern of antibiotic resistance was determined during disk diffusion. Microplate and checkboard methods were investigated.

Results: The antibacterial effects of thyme essential oil against *Pseudomonas* were confirmed. In Kirby-Bauer method, up to 0.312 mg / ml halo, no growth inhibition was observed in bacteria. In the microplate dilution method, a concentration of 5 mg / ml was obtained as a result of MIC.

Conclusion: From the findings of this study, it was concluded that thyme essential oil can have significant antibacterial effects and subsequently its effects on the clinical and resistant strain of *Pseudomonas aeruginosa* can cause bacterial resistance failure and accelerate its treatment in the clinical ward.

1. Introduction

The Lamiaceae family is one of the largest plant families in the world (excluding the Arctic and Antarctic regions) and has about 200 genera and two to five thousand species of fragrant shrubs and short shrubs. Most mints produce terpenes and a variety of late compounds that store these compounds (mainly) in the epidermal glands of leaves, stems, and reproductive organs (Sheikholeslami, Mousavi, Ashtiani, Doust, & Rezayat, 2016).

Thyme (*Thymus vulgaris* L.) is a genus of dark mint plants that grows as dense shrubs on dry slopes and between boulders in various Mediterranean regions, including France, Portugal, Spain, Italy and

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Greece. This plant exists in semi-arid areas of New Zealand for several thousand hectares by car. This species has not been seen wild in our country. Of course, thyme is widely grown every year in Spain, Germany, France, Portugal, the United States, the Czech Republic, Slovakia, Hungary and North Africa. In Iran, the area under cultivation of this species is increasing (Farahani, Barati, Arjomandzadegan, & Vatankhah, 2020).

Thyme is used in a variety of food, pharmaceutical, health and cosmetic industries. Thyme oil has antispasmodic, windbreak, antifungal, disinfectant, anti-worm, anti-dermatitis and expectorant properties. Thyme essential oil is one of the ten famous essential oils that have antibacterial and antifungal properties, antioxidants, natural food preservatives and anti-aging mammals and has a special place in world trade. Thyme is also used in a variety of foods and is used as an aromatic compound in most important food products such as beverages and dairy desserts. Although the growth, quality and quantity of active ingredients in medicinal plants, including dry matter accumulation and essential oil biosynthesis, are primarily controlled by genetic processes, environmental factors also play an important role. What is important is that thyme oil, which is cultivated in different regions, is different in terms of color, taste, viscosity and chemical composition, and seasonal variation has a significant effect on its yield and oil composition (Mottaghiyan, Aghazadeh, Mahmoodzadeh Hosseini, & Imani Fooladi, 2019). Agronomic factors also affect the quantitative and qualitative yield of thyme, which is the planting distance from important agronomic factors that have a significant effect on the yield of essential oil and dry matter per unit area. It was found that shorter plant spacing significantly increased the yield of the branch head and the amount of essential oil, with a planting distance of 5 cm having the highest yield.

Europe, along with the United States, is one of the major consumer markets for thyme. Trade statistics show that the United States imports about 1,000 tons of thyme annually. 90% of thyme oil in world trade is produced in Spain (Farahani et al., 2020).

Pseudomonas bacteria and its characteristics

The common ancestors of the pseudomonad lived hundreds of millions of years ago, yet they were classified by humans in the late nineteenth century. The common name *Pseudomonas* was coined by Walter Migula from 1894 to 1900 as a genus (Chouhan, Sharma, & Guleria, 2017) of gram-negative, rod-shaped, polar, and flagellate bacteria. (false) and monas means a single unit that can give the meaning of a false unit (false unit).

2. Methods

Nutrient agar medium of German Merck company, Blood agar medium of German Merck company and McConkey agar medium of German Merck company and Müllerhinton agar medium of German Merck company were used. The type of bacteria used was *Pseudomonas* and sheep blood was used for blood agar. To perform the tests, the antibiotic discs of chloramphenicol, gentamicin, ampicillin, ciprofloxacin and imipenem, p-paracillin were used. Microliters of thyme essential oil were inoculated on the discs (Basavegowda, Patra, & Baek, 2020; Cunha, 2018; Narasagoudr et al., 2020; RANI, RAO, & SUKUMAR).

The MIC test was performed on a plate of 96 sterile cells using the microdilution broth method (Sardarodiyani, Arianfar, Sani, & Naji-Tabasi, 2019). First, 100 μ l of Müllerhinton culture medium was poured into 96 microplate wells. Then 100 μ l of essential oil was added to the first well of each row and diluted from the second to the third house and so on until the ninth house. Finally, 100 μ l of diluted microbial suspension was added to all wells equivalent to half a McFarland tube. After 24 hours of incubation at 37 ° C by a tray-reading stand base made for this purpose, the bottom of the plate was

observed in the mirror under light. We recorded the presence of turbidity, which indicated the growth or non-growth of bacteria, in a special table. According to the concentration definition, the last (thinnest) well was placed in which no turbidity was created. Essential oil control house, culture medium and microbial equivalent MIC were also considered separately (Sardarodiyani et al., 2019).

3. Results

The results of the study of the susceptibility of *Pseudomonas* resistant bacteria to thyme essential oil and breaking the resistance of this bacterium are in two stages. In the first stage, the results of the studies performed on the bacterium are presented and the degree of susceptibility is observed. We examine the treatment of rats infected with *Pseudomonas*.

Initially, the *Pseudomonas* bacterium isolated from the cystic fibrosis patient, despite clinical tests, was tested for antibiogram by disc diffusion method in the treatment center. The results of determining the antibiotic susceptibility of the isolated bacterium were as follows: the bacterium was evaluated by ciprofloxacin, piperacillin, imipenem, amikacin, polymyxin B, gentamicin disks, and the obtained growth inhibitions were matched with the CLSI table and the growth inhibition halos were presented as follows (Table 1)

It was observed to be sensitive only to imipenem and semi-sensitive to amikacin and resistant to other antibiotics.

Table 1: Results of *Pseudomonas* bacterium diffusion disk and conformity of halos to CLSI table

Antibiotic disc	CP	PIP	Polymyxin B	AN	GM	IPM
Aura obtained	14	15	5	14	10	24
	S: >21	S: >21	S: >12	S: >17	S: >15	S: >19
interpretation according to CLSI table	I: 16-20	I: 16-20	I: --	I: 15-16	I: 13-14	I: 16-18
	R: <15	R: <14	R: <11	R: <14	R: <12	R: <15

As explained, to ensure bacterial susceptibility to thyme essential oil, microbial susceptibility tests were performed on isolated *Pseudomonas* bacteria. These tests include the Kirby Bauer-MIC method, the results of which are presented below.

Kirby Bauer Results:

In Kirby-Bauer method, the amount of growth inhibition halos in different concentrations was investigated and it was observed that in this method up to 0.312 mg / ml growth inhibition halo was observed for *Pseudomonas* bacteria (Figure 1).



Figure 1: Results of Kirby-Bauer method in bacteria.

Also, the results obtained from the microplate dilution method showed that, a concentration of 5 mg / ml was obtained as a result of MIC (minimum concentration inhibiting bacterial growth).

4. Conclusion

Pseudomonas aeruginosa is a gram-negative bacterium that is abundant in a variety of humid environments such as soil and water and can adapt to many other environments(Alalwan, 2018). This bacterium is also an opportunistic pathogen that infects patients in hospitals, especially burn wards. In these patients, the skin defense is lost. Burn wound healing is a complex process involving an early phase of sudden energy depletion and cellular necrosis that follows the stages of inflammatory phase, cell death, granulation tissue formation, matrix formation, and collagen rearrangement. Drugs that have anti-inflammatory, anti-inflammatory effects Bacterial or antioxidant activity are good candidates for wound healing. The compounds in the extract have all the above effects(Naz, 2018).

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The results of this study are consistent with previous studies. It is suggested that the extract has antibacterial effects by preventing bacterial growth and multiplication and can also defeat biofilm and its production(Valcourt, 2016).

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