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Antibacterial Screening of Oil of *Pistacia lentiscus* against *Staphylococcus aureus* ATCC No. 25923 in West of Iran

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Abstract

Ethnomedicinal plants are considered as recent resources for producing agents that could act as alternative to antibiotics in demeanor of antibiotic-resistant bacteria. *Pistacia lentiscus* (PL) is a native plant in Iran, and is used as a medicinal plant. The aim of the recent study is evaluation of antibacterial activities of PL against common pathogen (*Staphylococcus aureus* with ATCC No. 25923 (SA)) in Iran. The antibacterial effects of PL oil were evaluated by macro-dilution method in Mueller-Hinton broth medium, agar disk and agar well diffusion methods. The results revealed that the oil of PL inhibited the growth of SA and destroyed it. Also, when the concentration of oil was increased, the zone of inhibition was found to increase in many of the samples. In agar disk diffusion the widest inhibition zone of 13 mm occurred in 0.125 g/ml PL with no inhibition with DMSO. In agar well diffusion, values were lesser than the disk method. Inhibition ceased at levels of 0.003 g/ml PL. Minimum inhibitory and bactericidal concentrations of PL were 0.031 g/ml. The results defined that in tested bacterium, there was a considerable discrepancy in terms of sensitivity to PL oil. We believe that this study will provide support to the antibacterial properties of the oil. Our findings indicate the fact that the oil of PL can be useful as medicinal or preservative composition.

Keywords *Pistacia lentiscus*, *oil*, *Staphylococcus aureus*

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INTRODUCTION

Infections due to bacteria stay as a serious clinical difficulty. Antibiotics provide the primary basis for the treatment of microbial (bacterial and fungal) infections. But overuse of antibiotics is a primary cause for emergence and dissemination of multi-drug resistant bacteria[1]. Ethnomedicinal plants have been consumed as other remedies for the cure of different diseases[2-5]. Plants and spices are invaluable resources useful in daily life as food additives, flavors, fragrances, pharmaceuticals, colors or directly in medicine[6-8]. Plants possess antibacterial properties *in vitro*[9-12], and do not increase antibiotic resistance due to innate intrinsic anti-oxidative antimicrobial effects[13-15]. An oil is a concentrated hydrophobic liquid containing volatile aroma compounds from plants[16-18]. Oils could be extracted from different parts through distillation[19]. In recent years, interest in oils has increased due to their pharmacological properties which claim that oils have beneficial efficacy for the prevention, control and treatment of microbial disease[20-23]. Interest in oil of plants with antimicrobial properties has revived as a result of current problems associated with the use of antibiotics[24, 25]. In previous studies, it was demonstrated that oils are effective on a large numbers of Gram-negative and positive[26-28] bacteria.

PL is a species in the *Pistacia* genus and *Anacardiaceae* family. PL is cultivated in Iran as an ethnomedicinal plant. PL has many nutrients including polysaccharides, active proteins, essential amino acids, essential fatty acid, carotenoids, vitamins, and minerals. It is also used as a spice and food additive and it used in traditional medicine for gastrointestinal disorder and infectious disease[29,30]. Based on knowledge of authors, in comparison to many other medicinal plants, there is a very little data about antibacterial activity of PL oil collected from Iran. Hence, the aim of the current study was evaluation of antibacterial effects of the oil on PL with broth macro-dilution, agar well and disk diffusion methods.

2. MATERIALS AND METHODS

2.1. Oil extraction

A 20 g of dried PL was inserted in the sohxlet extractor. N-hexane solvent was splashed into three-neck- round bottom flask that is linked with the extractor and flask along with the condenser on the top to eschew any solvent losses. The lump

assembly was then placed on the temperature controller heater. After specified interval of the time the tryout was stopped and the trapped oil in the solvent was separated using rotary evaporator. The oil obtained after evaporation was weighed.

2.2. Source of microorganisms

Lyophilized *Staphylococcus aureus* with ATCC No. 25923 (SA) was provided by the Iranian Research Organization for Science and Technology activated on Tryptic Soy broth at 37°C for 18 h. Sixty 60µl of broth was transferred to Nutrient agar and incubated at 37°C for another 24 h; cell concentration was then adjusted to obtain final concentration of 10^8 cfu/ml using Muller Hinton broth.

2.3. Culture media

Mueller-Hinton Agar was accumulated according to the manufacturer's instruction (Oxoid, UK), autoclaved and dispensed at 15 ml per plate in 10 x 10 cm Petri dishes. All plates were incubated for 4 hours in 120 centigrade to ensure sterility before use.

2.4. Evaluation of antimicrobial activities

Agar disk and agar well diffusion tests were done to determine Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of PL to inhibit SA. The solution of PL yielded in 1g/ml from which six fold serial dilutions (v/v) of 60 µl were placed on each disk and well. DMSO and cephalexin were used as negative and positive controls, respectively. After 24 hours incubation diameters of growth inhibition zones around disks or wells were measured. For nomination of MIC, the macrobroth dilution method was used[31].

3. RESULTS

3.1. Agar disk diffusion test

In agar disk diffusion test, the widest zone was seen in 0.125 g/ml concentration (The value of growth inhibition zone was 12 mm in this dilution). No inhibition zone was observed due 0.003 g/ml concentration and DMSO. Growth inhibition zones due to different dilutes are listed in Figure 1.

3.2. Agar well diffusion test

With regard to PL, the widest zone was seen in 0.125 g/ml concentration (The diameter of growth inhibition zone was 10 mm in this dilution). There was no inhibition zone due to low concentration of 0.015, 0.007 and 0.003g/ml, respectively. No inhibition zone was observed due to DMSO. The data are discoverable in Figure 2.

Fig 1 The diameters of growth inhibition zones in agar disk diffusion test in different dilutions of PL.

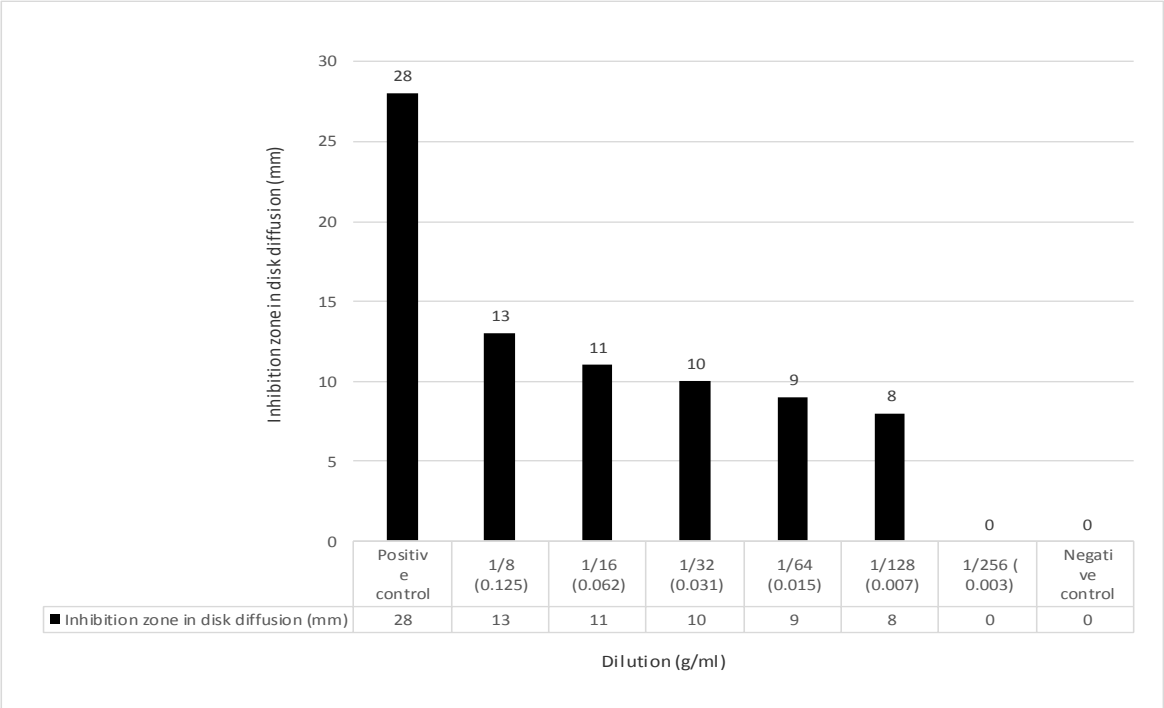
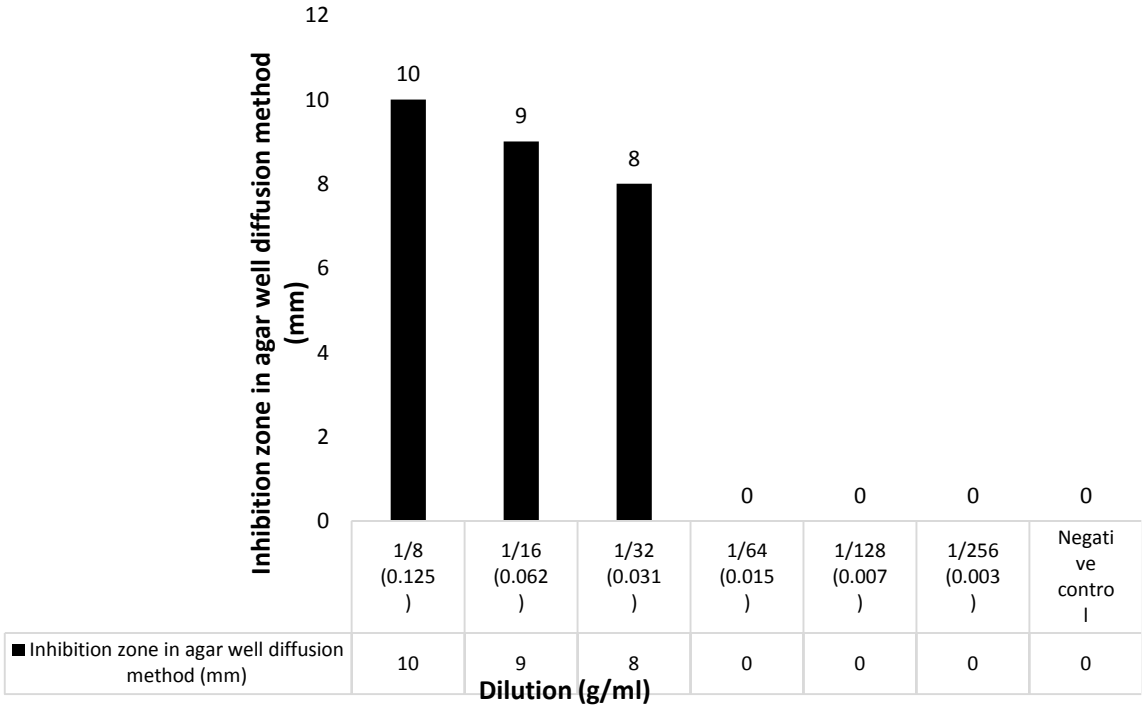


Fig 2 The diameters of growth inhibition zones in agar well diffusion test in different dilutions of PL.



3.3. MIC and MBC determination:

In the examined bacterium, MIC and MBC values were the same and equal to 0.031 g/ml concentration

4. DISCUSSION AND CONCLUSION

PL is an endemic and resistant species in dry and sub-dry forests in Iran. PL is one of the edible plants which have generated a lot of interest throughout human history as a medicinal panacea. A wide range of microorganisms have been shown to be sensitive to PL. It has been shown to be antiviral and antifungal, as well as possessing both antitumor and antithrombotic effects[29,30].

The antibacterial results demonstrated that the oil of PL inhibited the bacterium and the activities were considerably dependent upon concentration. Also, the results indicated that PL oil of 0.031g/ml concentration has prevented SA from the growth, also in this concentration it has destroyed this bacterium, actually MIC and MBC are equal for the bacterium. Thus, the research represents the antibacterial effects of the medical plant on PL. There are similarities between this study and the previous studies.

In several studies it was demonstrated that *P. lentiscus* have antibacterial activity against a panel of Gram-positive and Gram-negative bacteria[32]. Also it was found that MBC of oil of *P. lentiscus* in isolated clinical isolates of Helicobacter pylori was 0.002 g/ml[33]. In other studies it was reported that PL has strong antibacterial activity against *Escherichia coli*, *Staphylococcus aureus*, and BS[29, 34].

In this study, oil of PL have inhibited the growth of SA and eradicated it. Also, by increasing the concentration of the oil, the inhibition zone in many of samples increased. The results determined that in tested bacterium, there was a considerable discrepancy in terms of sensitivity to PL oil. In other words, the most sensitivity was observed in disk diffusion method. Our results support the use of the plant in traditional medicine and offer that oil of PL possesses good antibacterial properties. It can be used as antibacterial supplement in the developing countries towards the development of new therapeutic agent. Additional *in vivo* studies and clinical trials would be needed to justify. Also, further

assessment is incumbent on potential of the plant as an antibacterial agent in topical or oral applications.

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