

Study of the bactericidal and bacteriostatic effects of olive oil, sesame oil and their synergism on *Pseudomonas aeruginosa in vitro*

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ABSTRACT

Background and aims: Nosocomial infections are common problems in developing and developed countries. One of the pathogens that causes these infections is *Pseudomonas aeruginosa*. According to the antibiotal resistance of this bacteria, It is essential to find new treatments like new herbal medicine. This study was aimed to determine *in vitro* antibacterial effects of sesame oil, olive oil and their synergism on *P. aeruginosa*.

Methods: In this experimental study, first, olive oil and sesame oil were obtained by cold-pressing assay. Then, *P.aeruginosa* ATCC 27853 was prepared from Iranian Research Organization for Science and Technology. Oils and their mixture with the same proportion were made in concentrations from 2 to 1024 mg/ml and the antibacterial character of these oils on *P.aeruginosa* was investigated by broth micro dilution method and then the amounts of the MIC and the MBC were demonstrated.

Results: The MIC of olive oil, sesame oil and their synergism on *P.aeruginosa* were reported; 16 mg/ml, 128 mg/ml and 128 mg/ml and the MBC of these oils were indicated; 64 mg/ml, 512 mg/ml and 512 mg/ml, respectively.

Conclusion: This study showed that sesame oil, olive oil and their synergism can inhibit bacterial growth. Olive oil has more antibacterial effect on this bacteria than sesame oil and their synergism. So, we conclude that there was no synergistic effect in these oils.

Keywords: Sesame oil, Olive oil, Synergism, *Pseudomonas aeruginosa*.

INTRODUCTION

One of the most widespread and important causes of disability is nosocomial infections that increases the period of hospitalization, treatment funds, risk factors, and even mortality.

According to the reports, between 5 to 10 percent of patients admitted to hospitals in developed countries and 25 to 30 percent in developing countries involve in nosocomial infections, besides 100,000

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patients in developing countries pass away due to nosocomial infections.^{1,2}

Pseudomonas Aeruginosa has an important role in these kinds of infections. This bacteria is known as the third most common cause of nosocomial infections after *Staphylococcus aureus* and *Escherichia coli*.^{3,4} This bacteria is non-fermentative gram-negative bacteria that could be found in soil, water, plants, fertilizers and animal's skin. This bacteria is an opportunistic pathogen that widely lives in humid environments and causes urinary tract, eye, ear and wound infections and pneumonia which has a high incidence in some departments of hospitals.⁵⁻⁷

The resistance of bacteria such as *P. aeruginosa* to penicillin and most of beta-lactam antibiotics is a common problem, but it should be noted that this bacteria is sensitive to Antibiotics such as Piperacillin, Ciprofloxacin, and Tobramycin.⁸

Bacterial resistance to antibiotal treatments is one of the major global problems that causes implementation of new researches with the aim of finding alternative herbal medicine that has antibacterial properties and fewer side effects than the synthetic medicines. Different parts of plants such as their seeds, roots, bark, branches, leaves, flowers and buds have aromatic oils and liquids that naturally have antimicrobial properties. These properties are due to the presence of phenolic components in essences of plant. Administration of herbal medicines in some countries like China and India is so high while some other countries have lower administration. For example, in Iran, the ratio of these herbal medicines to other medicines is only 5:100.⁹

Olive oil and sesame oil are two vegetable oils that have antimicrobial properties and could be used as substitutions for antibiotics.^{10,11}

Olive (*Olea europea*) is a perennial and evergreen plant which could live up to 1000 years. Antioxidant substances such as phenolic components, tocopherols, pigments and unsaturated-bond fatty acids exist in Olive oil. Tocopherols have antioxidant features, and are considered in non saponifiable components. Sterols in olive oil also are anticancer.¹¹⁻¹⁴

Sesame (*Sesamum indicum*) plant is used in the pharmaceutical industry and is a dicotyledonous and gamopetalous plant. Because of its antioxidant, anti-inflammatory, and anti-apoptotic properties this plant is used in many fields like food industry. This oil contains lignans (sezamin, Sesamol and a little Sesaminol) that are antioxidant and protect body cells from being damage by free radicals and Oxidative stresses. This oil is used in treatment of burnt wounds.¹⁵⁻¹⁷

According to oxidative properties and the antimicrobial role of these oils in traditional medicine, the aim of this study was to determine the antimicrobial activity of sesame oil, olive oil and their combination on *P.aeruginosa*.

METHODS

In This study first the standard strains of *P.aeruginosa* ATCC 27853 was prepared from Iranian Research Organization for Science and Technology and According to instructions the bacteria were cultured. the following Biochemical tests (Gram Staining, oxidase, Oxidative/Fermentative, Catalase, Citrate, Nitrate Reduction, H₂S, Gelatin Hydrolysis, Cetrimide Test, Mannitol, Lipase, PigmentIndoletest) were done to ensure the purity of the strains.¹⁸ Olive oil and sesame oil were obtained by cold-press method. Since crude sesame oil is more stable than the refined one, we used crude sesame oil in this study.¹⁹

In the next step, the antibacterial properties of the oils on bacteria were examined by broth micro-dilution method. This means that olive oil, sesame oil and their synergism affected on *P.aeruginosa* separately in 96-well sterile plates based on Clinical and Laboratory Standards Institute(CLSI). This experiment was repeated three times for each sample.^{20,21}

To do bacteriological tests; sesame oil, olive oil and their synergism with the same proportion of them were prepared with concentrations of 2 mg/ml, 4 mg/ml, 8 mg/ml, 16 mg/ml, 32 mg/ml, 64 mg/ml, 128 mg/ml, 256 mg/ml, 512 mg/ml, and 1024 mg/ml. To perform the test after adding 100 micro liter of Mueller Hinton Broth to all wells, we added the oils with appropriate concentrations to the Mueller-Hinton broth and then 100 ml of suspensions 0.5 McFarland (1.5×10^8 CFU/ml) were added to wells. The first wells contained only bacterial suspension and Mueller-Hinton Broth (positive control) and second wells contained only Mueller-Hinton broth (negative control). After that, the

samples were incubated at 37 °C for 24 hours and then the optical density (OD) of wells, at a wavelength of 650 nm was collected by using a ELISA reader (Made in USA: State fax 2100). Then, all datas were analysed by using statistical analysis package SPSS statistical version 16.0. The lowest concentration that had no Turbidity in it was considered as the minimum inhibitory concentration (MIC) and to measure the minimum bactericidal concentration (MBC), free of turbidity wells were cultured on Mueller Hinton agar and then incubated for 24 h at 37 °C. The lowest concentration of the oils that bacteria did not grow in it, was considered as MBC.^{20,22}

RESULTS

While doing the experiments, the MIC and MBC of sesame oil, olive oil and their synergism for *P.aeruginosa* were measured and these amounts for olive oil are 16 and 64 mg/ml, for sesame oil are 128 and 512 mg/ml and for their synergism are 128 and 512, respectively (Figure 1).

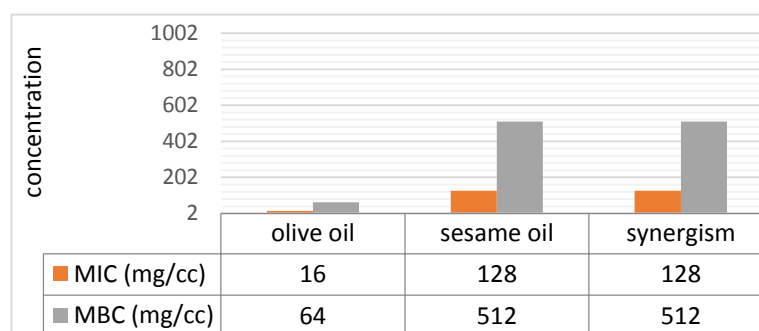


Figure 1: MIC and MBC amounts for olive oil, sesame oil and their synergism on *P. aeruginosa* bacteria

Based on the achieved findings, antimicrobial effect of these oils can be realized. This means that sesame oil, olive oil and their mixture showed growth inhibition and bactericidal effects.

Figure 1 shows a meaningful relationship between increasing of concentration of the oils and their antibacterial feature. We can also realize that in low concentration, olive oil has stronger antimicrobial effect than sesame oil

and for mixture of these oils, synergistic effect was not observed.

DISCUSSION

Researchers' attention to herbs is the reason of the wide researches on antimicrobial features of these plants. Nowadays, components which are generally harmless and named GRAS (Generally Regarded as Safe) have attracted a great deal of attention to themselves that among them biological compounds derived of plants can be mentioned.²³

In a research that determined the antibacterial effect of four olive (Shiraz, Oily, Yellow, and Dezul) extracts on *Bacillus sureus* with Broth dilution method, reported the MIC of methanolic extract of Oily one 10 mg/ml, which shows more antimicrobial activity of these extracts on the *B.sureus* than olive oil on *P.aeruginosa* (in this present study).²⁴

In a study that evaluated the antibacterial effect of Basil extract on *P.aeruginosa* by determining the diameter of inhabitation zone, reported the diameter of inhabitation zone for the concentrations 25, 50, 100, and 200 mg/ml; 0, 4, 8 and 12 mm respectively, while in the present study, that was done with broth micro dilution method, MIC amounts for olive oil, sesame oil and their synergism were 16, 128 and 128 mg/ml, respectively.²⁵

In another research, which studied the antimicrobial activity of alcoholic and aqueous extracts of common hop (*Humulus lupulus*) and oak (*Quercus castaneifolia*) on *P.aeruginosa*, called MIC of alcoholic and aqueous extracts of common hop 128 and 512 mg/ml and for oak 250 and 512 mg/ml, respectively. These findings were obtained by broth macro dilution and agar diffusion methods; it can be concluded that sesame oil, synergism of sesame oil and olive oil in the present study have almost the same antibacterial effect with alcoholic extract of

common hop, while they have more antimicrobial activity than aqueous extract of common hop and extracts of oak. Olive oil also has more antibacterial feature than alcoholic and aqueous extracts from both plants.²⁶

In a study that started evaluating the antibacterial activity of Coriander on *P.aeruginosa* with micro plate method, indicated MIC and MBC of aqueous extract of coriander plant on this bacteria 50, 100 mg/ml and ethanolic extract 25, and 50 mg/ml, respectively. By comparing these results with results of the present study, it can be found out that olive oil in comparison with alcoholic and aqueous extracts of coriander has more growth inhibition but this oil has less bactericidal effect than ethanolic extract, although sesame oil and the synergism of sesame oil and olive oil have less antibacterial effect than these two extracts.²⁷

According to the past studies, olive oil and sesame oil show different therapeutic characters which is a consistent point with current study which indicates their antibacterial features on *P.aeruginosa* but the most important point of this study was focusing on the synergistic characters of sesame oil and olive oil, although the results show that the mixture of these oils doesn't have synergistic effect.

CONCLUSION

According to the results of this study, it could be concluded that olive oil in comparison with sesame oil and also their synergism, has more growth inhibitory and bactericidal effects. Also, the evaluation of the findings showed that there was no synergic effect in these two oils. In other word, the mixture of these two oils did not show exclusive antimicrobial feature than each oil individually. Eventually, we suggest to diagnose and refine the substances which have important role in antibacterial

properties of these two oils, so that they could be used in treating of severe infections. Also, the use of synergism of these two oils with other oils should be investigated or antibacterial feature of synergisms with different proportions can be surveyed.

CONFLICT OF INTEREST

There is no conflict of interest.

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