

Evaluation of antibacterial effect of sesame oil, olive oil and their synergism on *Staphylococcus aureus in vitro*

Reza Heidari-Soureshjani^{1*}, Zia Obeidavi², Vahid Reisi-Vanani¹,
Sepehr Ebrahimi Dehkordi¹, Neda Fattahian¹, Abolfazl Gholipour³

¹Students Research Committee, Shahrekord University of Medical Sciences, Shahrekord, I.R. Iran; ²Students Research Committee, Lorestan University of Medical Sciences, Khorramabad, I.R. Iran; ³Public Health Dept., Shahrekord University of Medical Sciences, Shahrekord, I.R. Iran.

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ABSTRACT

Background and aims: Nosocomial infections are the main cause of the problems related to health and health care costs. One of the pathogens that causes these infections is *Staphylococcus aureus*. Its resistance to antibiotics forced researchers to find herbal substitution such as Sesame and Olive. This study was aimed to determine antibacterial effect of sesame oil, olive oil and their synergism on *S. aureus*.

Methods: In this experimental study, at first, olive oil and sesame oil was extracted by cold press assay and the mixture of them was prepared with equal proportion of both oils, and then the standard strain of lyophilized *S. aureus* ATCC 25923 was prepared from the Iranian Research Organization for Science and Technology. To determine minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the oils, micro-broth dilution method was used.

Results: This study indicated the amounts of MIC for sesame oil, olive oil and their synergism; 32 mg/ml, 32 mg/ml, 8 mg/ml and the amounts of MBC; 128 mg/ml, 128 mg/ml, 32 mg/ml, respectively.

Conclusion: The results showed the same antibacterial activity for olive oil and sesame oil. It also showed that mixture of these oils enhances the growth inhibitory and bactericidal properties of the oils compared to using them individually.

Keywords: *Staphylococcus aureus*, Olive oil, Sesame oil, Synergism.

Original article

INTRODUCTION

Nowadays, infectious diseases are the second cause of death in the world.¹ These infections are followed by exorbitant expenses of treatment and increase in mortality and the outbreak of the infection.

According to statistics, nosocomial infections are the main cause of the death of 88 thousand people in the world and the reason of 4.5 billion economic losses in a year.² One of the common bacteria

*Corresponding author: Reza Heidari-Soureshjani. Students Research Committee, Shahrekord University of Medical Sciences, Shahrekord, I.R. Iran, Tel: 00989138807195, E-mail: heidari.reza1995@gmail.com

involved in nosocomial infections, which hospitalized patients infected continually, is *Staphylococcus aureus*.³

S. aureus is a gram-positive Cocci bacteria which could produce pestiferous toxic factors, it is considered in pathogens that interface between humans and animals. *S. aureus* can survive on dry surfaces for months and infects its host from skin, mouth and nose. Its major hosts are humans and the percentage of its incidence in human is about 30%. According to a report from world health organization (WHO) this bacteria is the most common cause of the post-operative wound infections.⁴⁻⁸ Researches show that this bacteria is the cause of many skin infections and diseases such as pneumonia, meningitis, osteomyelitis, endocarditis and bacteremia.⁹

Resistance to antibiotics could be seen in many bacteria such as *S. aureus*, For example some strains of this organism are resistant to aminoglycoside antibiotics, methicillin and primary antibiotic like penicillin. This antibiotic resistance is growing continually, and this problem is related to the patient's duration of hospitalization.^{3,10,11}

In recent years, due to the side effects and drug resistance of bacteria, herbal medicines are used as a substitutes.¹² Although about 25 to 50 percent of drugs are gained from plants and the usage of medicinal plants has been increased, it still is needed to pay more attention to these ways of struggling against bacteria.¹³

One of these herbs is Sesame (*Sesamum indicum* L.) that is a flowering plant in the genus *Sesamum* and the family of Pedaliaceae. Sesame is an annual plant and native to tropical areas. 50% of a sesame seed consist of oil, containing Sesamol, Sesamol, Sesamin, Sesaminol, γ -tocopherol and polyphenols, Excellent stability of this oil against oxidation is the reason of its usage as a solvent in

pharmaceutical industry.¹⁴⁻¹⁷ This oil is a rich source of non-saturated fatty acids like oleic acid and linoleic acid with percentage of 39.09% and 40.39%, respectively.¹⁸ An important feature of this oil is its anti-bacterial and anti-inflammatory properties. So, it has many usages, like treating superficial burns and also it has an important role in traditional medicine.¹⁹

Olive tree (*Olea europea* L.) is another herb, it grows in tropical and subtropical regions and it has been used for medical purposes since many years ago.^{20,21} High consumption of olive oil as a rich source of lipids enhanced people's health in the Mediterranean regions.^{22,23}

This oil contains phenolic and polyphenolic compounds such as oleuropein and hydroxytyrosol that eliminate free radicals. Olive oil contains fat-soluble vitamins, especially vitamin E, which is one of the most important protective factors against free radicals. It has antimicrobial, anti-inflammatory and antioxidant activity and is used topically to treat skin diseases.²⁴⁻²⁷

Synergism of vegetable oils with no changing in chemical and biological processes, could alter their physicochemical properties and increases their antioxidant activity.²⁸

Since *S. aureus* is resistant to a variety of antibiotics, Using plant sources such as olive oil and sesame oil with their antimicrobial properties is important. So, this study was aimed to determine *in vitro* antibacterial effect of olive oil, sesame oil and their synergism on *S. aureus*.

METHODS

In this experimental study, at first, the standard strains of lyophilized *S. aureus* ATCC 25923 were provided by the Iranian Research Organization for Science and Technology. After culture, some biochemical tests (coagulase, catalase,

Dnase) were done to ensure purity of strains. Then, bacterial suspensions based on standard 0.5 McFarland (1.5×10^8 CFU/ml) was prepared.²⁹

For preparation of olive oil and sesame oil, cold pressing method was used. It should be noted that sesame oil was crude because crude oil, which is obtained by cold pressing method is more stable than the refined oil.

In the next step, antibacterial properties of oils were evaluated by micro-broth dilution method. This means that olive oil, sesame oil and their synergism conducted separately in 96-well sterile plates on *S. aureus*, based on the Clinical and Laboratory Standards Institute in triplicate for each sample.³⁰

To do bacteriological tests, sesame and olive oils at 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, 1024 mg/ml; synergism of them with equal proportions of sesame oil and olive oil at similar concentrations were prepared. Then, 100 μ L of Mueller Hinton broth was introduced into each well. After that, concentration of each oil added to Mueller Hinton broth. Afterwards, 100 μ L of bacterial suspension, equal to 0.5 Mc

Farland added to the wells. The first well contains only bacterial suspension and Mueller Hinton Broth (positive control) and second well containing Mueller Hinton broth and oil (negative control). After that the samples were incubated at 37 °C for 24 h. Then, optical densities of the samples were read at 650 nm wavelength with an ELISA reader (State Fax 2100, USA).

To determine the minimum inhibitory concentration (MIC), concentrations of the last wells that did not have any turbidity (the lowest concentration) was considered MIC. To determine the minimum bactericidal concentration (MBC), all wells without turbidity were cultured on Mueller Hinton agar and then incubated for 24 h at 37 °C. The lowest concentration of oil that bacteria did not grow in it was considered as MBC.³⁰

RESULTS

Determination of MIC and MBC of the oils against *S. aureus* was measured with micro-broth dilution technique, and indicated the amount of MIC for sesame oil, olive oil, and their synergism; 32 mg/ml, 32 mg/ml and 8 mg/ml; and also their MBC; 128 mg/ml, 128 mg/ml and 32 mg/ml, respectively (Table 1), (Figure 1).

Table 1: Pattern of *S. aureus* Sensitivity

| Oils | Concentration (mg/ml) | | | | | | | | | |
|-----------|-----------------------|---|---|----|----|----|-----|-----|-----|------|
| | 2 | 4 | 8 | 16 | 32 | 64 | 128 | 256 | 512 | 1024 |
| Sesame | - | - | - | - | + | + | ++ | ++ | ++ | ++ |
| Olive | - | - | - | - | + | + | ++ | ++ | ++ | ++ |
| Synergism | - | - | + | + | ++ | ++ | ++ | ++ | ++ | ++ |

(-): indicates bacterial growth, (+): indicates inhibition of growth, (++) : indicates the bacteria has been killed.

According to the results MIC and MBC of olive oil and sesame oil are similar, but their synergism has more antibacterial

activity than olive oil and sesame oil individually. These results indicate that the mixture of these oils has a positive synergy.

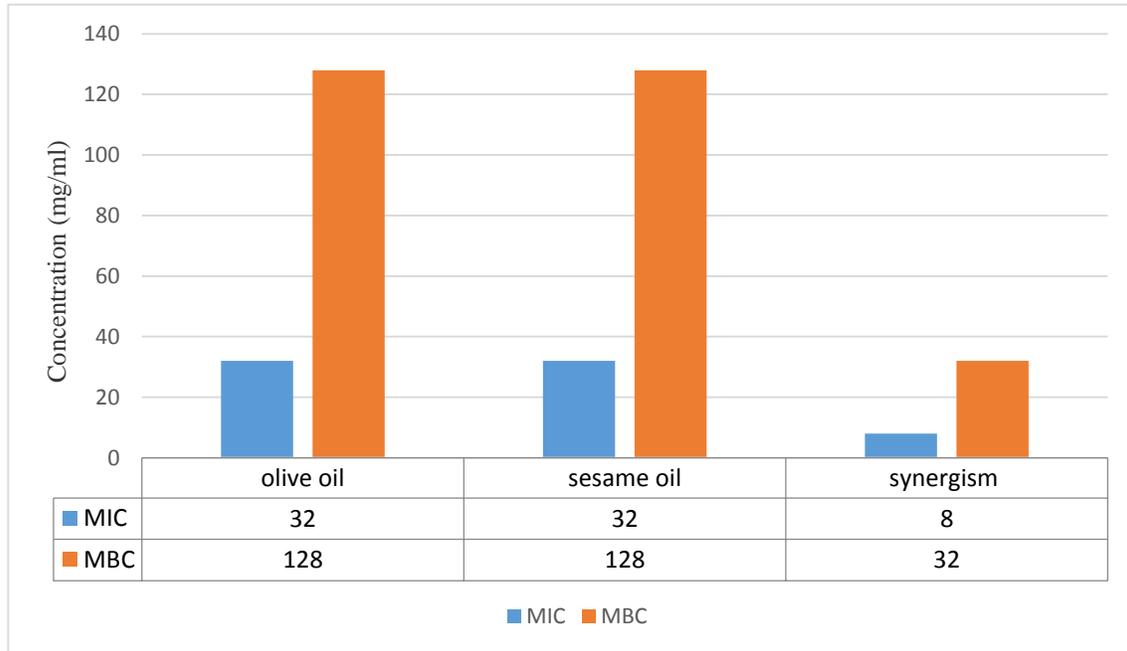


Figure 1: MIC and MBC of olive oil, sesame oil and their synergism.

DISCUSSION

S. aureus is a human pathogen. This bacteria is the cause of many infections and its resistance to antibiotics, which is a fundamental problem in medical sciences, has made researchers focus on finding new ways to deal with this problem.^{6,12}

In a research that evaluated the antibacterial effect of methanolic extract of Rosemary on *S. aureus*, reported MIC and MBC of this extract, 156 mg/ml and 312 mg/ml, respectively.³¹ The comparison of these findings with current study shows that the extract of rosemary has less antibacterial effect on *S. aureus*.

A study on *S. aureus* indicated the aqueous and ethanolic extract of henna has an antibacterial effect on *S. aureus*. Findings that were gathered by agar diffusion method

declared MIC of aqueous and ethanolic extract of henna 2.5 mg/ml and 3 mg/ml, respectively. In this research MICs of the extracts were equal to their MBCs, which shows strong antibacterial property of henna in comparison with olive oil and sesame oil.³² Since the amounts of MIC and MBC of henna is much lower than the amounts obtained for olive oil and sesame oil in present work, it can be understood that henna is more antibacterial than olive oil and sesame oil.

In another study that has examined the antibacterial properties of sesame seeds lignans on *Bacillus cereus*, *S. aureus* and *Pseudomonas aeruginosa*, the results showed that Sesamol (one of sesame seed lignans) in the concentration 2 mg/ml is able to stop the

growth of *B.cereus* and *S. aureus* completely, and inhibit 80% of *P.aeruginosa*'s growth.³³ As it could be seen, amounts of MIC obtained in this study are lesser than the MIC obtained in current study. The reason of this is probably studying only one of sesame seeds lignans, but we investigated sesame oil (consists of several lignans).

In a study that was done in Italy, the effect of oleuropein and hydroxytyrosol obtained from olive oil was studied. MIC amounts of hydroxytyrosol and oleuropein gained with micro-broth dilution method on *S. aureus*, were 0.00758 mg/ml, 0.0625 mg/ml, respectively. That shows growth inhibitory properties of these compounds.³⁴ The advantage of this study is that it has evaluated Oleuropein and hydroxytyrosol (important antibacterial component of olive oil) separately; However, in present work the main focus was on anti-bacterial effect of synergism of olive oil and sesame oil (not one or more specific compounds).

Another study on sesame oil, canola oil and their synergism indicated that synergism of these oils has more antioxidant activity and stability than canola oil, and in the present research, the antibacterial effect of the combination of these oils was higher than each one individually.²⁸ The reason of it might be the increasing diversity of phenolic compounds in the mixture of oils.

CONCLUSION

Finding of this study indicated that not only sesame oil and olive oil have inhibitory property on growth of *S. aureus*, but also they could kill these bacteria in higher concentration completely. It also showed the synergistic effect of the oils which means that synergism of the oils has more antibacterial effect. Due to the fact that *S. aureus* causes infections in human mostly through skin and skin ulcers, by improving their antibacterial effect these oils could be

used in medicinal purposes like using them in burn ointment.

It was not possible in this study to investigate side effects of the oils and this was one of its limitations. So, we suggest to culture human cells besides bacteria and evaluate oils' effects on them.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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