

## Bactericidal and Bacteriostatic effect of sesame oil, olive oil and their synergism on *Escherichia coli in vitro*

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### ABSTRACT

**Background and aims:** *Escherichia coli* is one of the important bacteria in nosocomial infections and its resistance to a wide range of antibiotics caused many problems for doctors and patients. So, it was attempted to find new substitution for these antibiotics. The aim of the present study was to determine the antimicrobial effect of olive oil, sesame oil and their synergism on *E. coli*.

**Methods:** In this study, olive oil and sesame oil were extracted by cold press assay and standard strain of lyophilized *E. coli* ATCC 25922 was prepared from the Iranian Research Organization for Science and Technology. Olive oil, sesame oil and their synergism, with the same proportion, in concentrations of 2 mg/ml to 1024 mg/ml were made individually and their MBC and MIC amounts were determined by broth micro dilution.

**Results:** This study declared MIC and MBC for olive oil; 16 mg/ml and 64 mg/ml, for sesame oil and their mixture; 64 mg/ml and 265mg/ml, respectively.

**Conclusion:** The study showed that both oils and their synergism inhibits the growth of bacteria and the antibacterial properties of sesame oil is approximately equal to the mixture of olive oil and sesame oil and although the oils have good antibacterial effect, there was no synergetic effect in these oils.

**Keywords:** *Escherichia coli*, Olive oil, Sesame oil, Synergism.

### INTRODUCTION

Despite significant medical advances in the past decade, nosocomial infections have high prevalence. These kinds of infections are such important problems in developing and developed countries.<sup>1,2</sup> Bacteria, like

*Escherichia coli*, are one of the microorganisms that cause these infections.

*E. coli* is a Gram-negative bacilli bacteria in the family Enterobacteriaceae which normally live in the intestines of

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humans, animals, water, soil and living plants.<sup>3,4</sup> This pathogen has an important role in urogenital infections which involve 4 million women in the United States each year.<sup>5,6</sup> Other nosocomial infections caused by these bacteria are bladder infections, wound infections, gastroenteritis, neonatal meningitis and sepsis. The discovery of antibiotics was an important step in dealing with pathogens, but using them in excess, lead to the appearance of bacterial resistance mechanisms. An example of this antibiotic resistance is *E. coli*'s resistance against beta-lactam antibiotics.<sup>4,7,8</sup> The increasing use of ineffective chemical medicines which is associated with increase in bacterial resistance, has led researchers to look for alternative herbs that have fewer side effects and cost.<sup>9,10</sup> Herbal medicine, which is so important in traditional medicine, is the source of one-third of all the administered drugs.<sup>11</sup>

One of useful herbs in traditional medicine is olive (*Olea europaeae*). This plant is in the family Oleaceae and evergreen tree, with long life. Scientists have attributed the origin of olive in Asia or the Mediterranean regions.<sup>12,13</sup> Olive has anti-microbial, anti-oxidant and anti-inflammatory properties. Phenolic compounds are the main cause of these medicinal properties. This plant has many phenolic compounds like hydroxytyrosol, oleuropein and other components with anti-microbial effects.<sup>14,15</sup> Another useful herb is sesame (*Sesamum indicum*) which is one of the oldest and most well-known crops in the world and its origin is in Africa.

Sesame oil is very popular in Asia and is commonly used in Africa and Asia's medicine to treat wounds, especially burns and is also used as an antidote to poisons. It also has oral intake and has antimicrobial properties.<sup>16-18</sup> Sesame seed contains 20% proteins and 50% oil which contains vitamins E and Unsaturated fatty acids.<sup>19</sup> Sesame is a good source of antioxidants like

Sesamol, Sesamolol and Sezamin.<sup>20</sup> Mixing oils is a desirable strategy to increase their antioxidant properties.<sup>21</sup> Both oils have important anti-bacterial effects that have been approved, but there was not any good study about their synergism's properties as result. In the current study, it was aimed to determine anti-bacterial effect of these oils and their mixture on *Escherichia coli*.

## METHODS

In this experimental study, at first the standard strain of *E. coli* ATCC 25922 was bought from Iranian Research Organization for Science and Technology and then after culturing them, it was performed some biological tests (Catalase, Oxidase, Lactolase) to ensure their purity. It was prepared bacterial suspension based on 0.5 McFarland ( $1.5 \times 10^8$  CFU/ml).<sup>22</sup> It was prepared the oils by cold-pressing assay as refining sesame oil reduces its stability.<sup>21</sup> We determined anti-bacterial effect of the oils with micro-broth dilution method.

Sesame oil, olive oil and their mixture conducted in 96-well sterile plates on *E. coli* separately, based on the Clinical and Laboratory Standards Institute (CLSI) in triplicate for each sample.

To do bacteriological tests, it was prepared olive and sesame 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; Then it was also made mixture of them with the same proportions of both oils at similar concentrations. To do the test, it was added 100  $\mu$ L of Mueller Hinton broth to each well and then it was add appropriate oils to the mediums and finally added 100  $\mu$ L of bacterial suspension (equal to 0.5 McFarland) to them. The first well contains Mueller Hinton broth and oil (negative control) and second well contains only bacterial suspension and Mueller

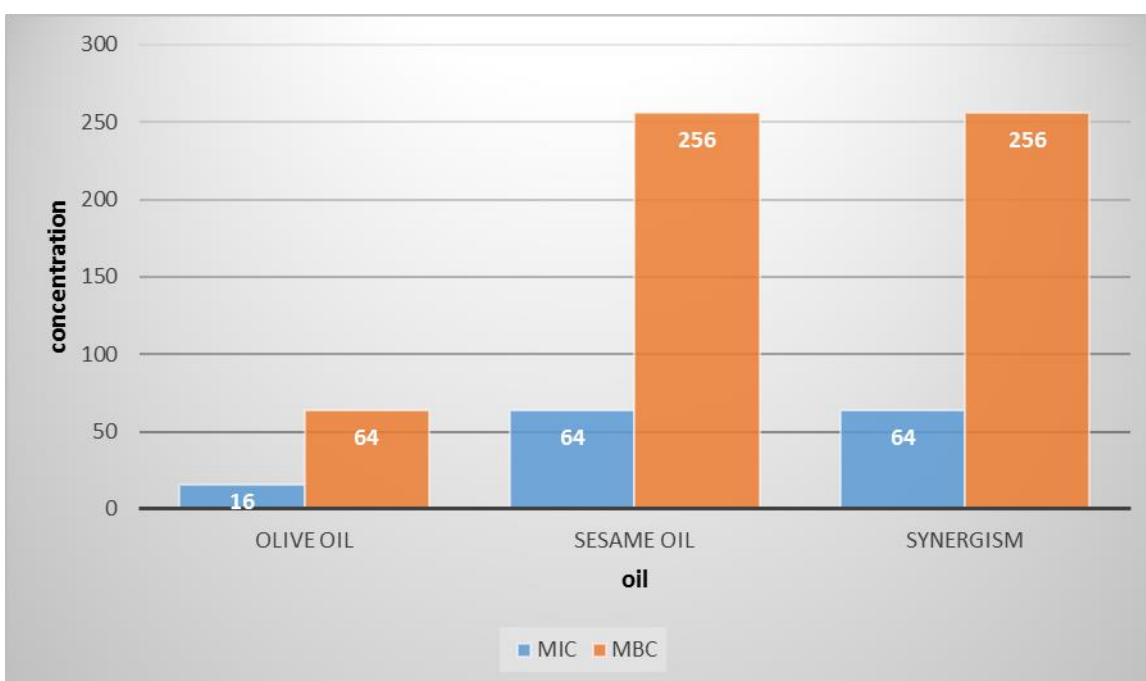
Hinton Broth (positive control). Then, it was incubated the samples at 37 °C for 24 h. after that, it was read optical densities of them at 650 nm wavelength with an ELISA reader (State Fax 2100, USA).

It was determined 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 blood

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.<sup>23</sup>

## RESULTS

Based on the results obtained in this study the amounts of MIC and MBC of olive oil on *E. coli* were 16 mg/ml and 64 mg/ml. These amounts for sesame oil and their mixture were approximately the same and 64 mg/ml and 256 mg/ml, respectively (Figure 1).



**Figure1:** MIC and MBC amounts for sesame oil, olive oil and their synergism on *E. coli*

As it could be seen, there is meaningful relationship between increasing of concentrations of the oils and reduction in the growth of bacteria, so that by increasing the concentrations, a significant reduction in bacterial growth is detectable. The lowest amount of MIC is for olive oil and this amount is approximately the same for sesame oil and their mixture, In another word, between sesame oil, olive oil and their synergism; olive oil has the most antibacterial effect on *E. coli* and the

amounts of its MIC and MBC is 4 times lesser than these amounts for sesame oil and their synergism. As the results show, there isn't synergistic effect in the mixture of these oils.

## DISCUSSION

In a study that evaluated the effects of methanol extracts of plants from 40 different families on *E. coli* by agar diffusion method and measuring the zone of inhibition. The

results showed that cloves and cinnamon had good antimicrobial effect on this bacteria.<sup>24</sup> In our study, it was used broth micro dilution method which is more advanced method for measuring the antimicrobial properties of the oils. Another study was conducted on the antimicrobial properties of medical plants. In this study the antimicrobial properties of essential oil of *Mentha piperita* were gained by measuring the amount of its MIC and MBC on *E. coli*. This study reported the amounts of MIC and MBC for this plant 0.39 mg/ml and 0.78 mg/ml.<sup>25</sup> If we compare the results of this study to our study, it can be concluded that *Mentha piperita* has more antibacterial effect on *E. coli*.

In a study that evaluated antimicrobial effect of olive leaf extract against a broad spectrum of microorganisms. In this study, the amount of MIC and MBC for *E. coli* was 25 mg/ml and 50 mg/ml, respectively.<sup>26</sup> If we compare the amounts of MICs and MBCs of our study to this study, it can be concluded that extracts of olive leaf has more bactericidal effect on *E. coli* than olive oil although it has lesser growth inhibitory effect than the olive oil.

In a study that evaluated antibacterial properties of phenolic compounds in extract of olive leaf using macro broth dilution, the results showed that *E. coli*, *Bacillus cereus* and *Candida albicans* are the most sensitive microorganisms to extract of olive leaf.<sup>27</sup>

In a study that evaluated the properties of phenolic compounds like p-hydroxy benzoic acid, oleuropein, vanillic acid, and protocatechuic acid present in olive oil, in concentration 0.4 mg/ml, the results show that these compounds has antibacterial effect on *E. coli*. Researcher believes that these phenolic compounds are the main reason of olive oil's antimicrobial effect.<sup>28</sup>

In a study that evaluated the antimicrobial property of *sesame radiatum*'s oil on the same organism to our study with

agar disk diffusion method measured the zone of inhibition 10-19 mm.<sup>29</sup> This implies the effectiveness of the oil in killing *E. coli* and according to our study which was done by broth micro-dilution method (newer method), the antimicrobial effect of sesame oil was measured more accurately.

In another study on bactericidal properties of Lignans in sesame seeds on three bacterial species, the obtained results showed complete inhibition of the growth of *B. cereus* when facing the Sesamol in concentration of 2 mg/ml.<sup>30</sup> A study on synergism of sesame oil and canola oil showed that the mixture of these two oils is more antioxidant than each oil individually. In the current study, the mixture of the oils not only were not more antibacterial than each oil but also it had less antibacterial effect than olive oil.<sup>21</sup>

## CONCLUSION

The results of this study showed that olive oil, sesame oil and their synergism have growth inhibitory effects on *E. coli in vitro* and in high concentration, they could kill the bacteria. As a result, these oils and their phenolic compounds could be used in producing herbal medicines against bacterial infections. It is suggested to refine the oils and used the phenolic compounds in high dosages for treating infections.

## CONFLICT OF INTEREST

There is no conflict of interest.

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