Research Article

# The synergistic effect for sesame oil-ciprofloxacin on the sensitivity of some Enterobacteriaceae species

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**ABSTRACT**

**Background:** Hospital-acquired infections are important problems in developing countries with high prevalence rate. Enterobacteriaceae members regarded the most important pathogens among those responsible for such infections. Due to the high prevalence of antibiotic-resistant strains in pathogenic microorganisms that have emerged in recent decades, extensive studies have been conducted on the use of natural products and biologically active compounds extracted from medicinal plants. **Methodology:** This study was designed to determine the effect of sesame oil on increasing the sensitivity of ciprofloxacin (CIP) resistant bacteria where oil works synergistically with antibiotic as a bacteriostatic or bactericidal. **Results:** Thirty-two samples of people with urinary tract infections and diarrhea were isolated at Al-Ramadi Teaching Hospital and Private Laboratories, the different bacterial species isolated belong to Enterobacteriaceae included *Escherichia coli*, *Klebsiella*, *Proteus*, *Pseudomonas,* and *Salmonella.* The results showed the isolates converted from CIP resistance to sensitive when mix the sesame oil with CIP and the sensitivity increased with increased the concentration of sesame oil in *E. coli* (8–30 mm), *Klebsiella* (10–26 mm), *Proteus* (8–22 mm), and *Salmonella* (10–24 mm), respectively. **Conclusion:** The results of this study showed that there is an important effect of sesame oil synergistically with CIP on the Enterobacteriaceae resistant to this antibiotic and causing urinary tract infection and diarrhea and did not show the same effect when adding sesame oil or antibiotics separately.

**KEY WORDS:** Ciprofloxacin, *Proteus* spp., *Salmonella* spp., Sesame oil

## INTRODUCTION

Despite the great advances in medicine achieved in the past century, hospital infections have a high prevalence rate. These types of infections are important problems in developing countries.[1] Enterobacteriaceae are considered to be microorganisms that cause these infections, which are Gram-negative bacilli that may live normally in the intestines of humans and animals and are found in water, soil, and living plants.[2,3] These pathogens play an important role in urinary tract infections, which include 1 million women in the United States each year.[4] Other hospital infections caused by these bacteria are bladder infections, wound infections, gastritis, intestines, meningitis, and sepsis.[5] The discovery of antibiotics was an important step in dealing with pathogens, but their excessive use led to the emergence of new bacterial resistance mechanisms, for example, the resistance of Enterobacteriaceae to ciprofloxacin (CIP).[6,7] CIP is a second-generation fluoroquinolone antibiotic that acts as a lethal agent for bacteria by replication inhibiting the DNA gyrase and Topoisomerase IV, thereby inhibiting DNA replication and reproduction.[8,9] Sesame (*Sesamum indicum*) belongs to the family Pedaliaceae, It is one of the oldest and most famous crops in the world and originates in Africa.[10] It is considered a reputable herbal remedy in Asia and Africa, used as a treatment for wounds, burns, antitoxins, and inflammation of mouth membranes and has antimicrobial properties.[11,12] Sesame seeds contain 20% protein and 50% oil containing Vitamin E and unsaturated fatty acids.[13] Gas chromatography–mass spectrometry phytochemical screening of sesame oil showed the presence of carboxylic acid and phenol groups, especially some of the most potent antioxidants such as sesamol, sesamolin, and sesamin.[14,15] According to oxidative properties and the antimicrobial role of this oil in traditional medicine, this study aimed to determine the antimicrobial activity of sesame oil synergy with antibiotic on CIP-resistance Enterobacteriaceae.

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## EXPERIMENTAL SECTION

### Isolation and Diagnosis of Bacteria

In this experimental study, samples were collected from patients with urinary tract infection and diarrhea in Al-Ramadi Teaching Hospital and Private Laboratories, after culturing them in different media such as blood agar, MacConkey agar, S.S. agar, and Nutrient agar, these media were prepared according to the company's instructions for each one for the studying the cultural characteristics of bacterial colonies in these media. Then, some biochemical tests were conducted such as oxides, catalase, and indole test to ensure purity and to make sure the isolates were diagnosed with Vitek 2.

### Sensitivity Test for Isolates

The sensitivity test for bacterial isolates was carried out with three replications for each isolate by agar diffusion method and the CIP-resistant isolates were selected until the effect of sesame oil will test later.

### Preparation of Sesame Oil

Local sesame oil was supplemented by the special plant to produce sesame oil in Al-Anbar/Iraq, where it was obtained purely.

### Preparation of Sesame Mixture

Sesame mixture is prepared by adding 60 ml of sesame oil to 20 ml of dimethyl sulfoxide (DMSO), which is a polar solvent and non-lethal of bacteria. It dissolves sesame oil to form an emulsifier that blends easily with the prepared broth culture media to grow bacteria and treat it with sesame oil and keep it in a tightly sealed container for use later.

### Test the Effectiveness of Oil Sesame Oil

The bacteria were treated in two ways: The first by adding oil to the antibiotic discs when testing the sensitivity and the second by adding the oil to the media before germination of the bacteria; then, the sensitivity test and the addition of antibiotics as follows:

#### *First method: Agar diffusion method*

This was done by culturing (active isolates for 1 h in nutrient broth) in Muller–Hinton agar by agar diffusion method and placing of CIP antibiotic discs and then adding crude sesame oil to the antibiotic disc of different sizes (10, 15, and 20 µl) with three replicates and incubating at 37°C for 18 h, then the diameter of inhibition zone was measured. This result compared to the control.

#### *Second method: Ensure the solubility of oil in the microbial medium*

This method was performed using 10 ml clean sterile tube (7, 8, 9, and 10 ml) of the Mueller-Hinton broth and completing the size of each tube of mixture (sesame oil + DMSO) to be 3, 2, and 1, respectively, with three replicates per sample, while this mixture was not added to the 10 ml tube from the medium and was considered as a negative control. The bacterial suspension (0.5 McFarland) was added to the prepared medium in the tubes and incubated at 37°C for 18 h, the sensitivity test for the developing isolates was then carried out by agar diffusion method and incubated for 18 h at 37°C. The diameter of the inhibition zone was measured in millimeter by the ruler and in comparison with control isolates; bacteria that were converted from CIP resistance were isolated into a sensitive.

## RESULTS AND DISCUSSION

The results showed that the positive bacterial culture was 87%. The bacteria were isolated using blood agar and MacConkey agar and (121) bacterial isolates were obtained, including *Escherichia coli*, *Klebsiella pneumonia*, *Salmonella*, *Proteus mirabilis*, and *Pseudomonas aeruginosa*. *E. coli* bacteria were the most common causes of infection and the number of isolates was (45) isolation by (37%), while *K. pneumonia* ranked second with 37 isolates (31%). *Salmonella* spp., 21(18%); *P. mirabilis*, 10 (8%) and finally *P. aeruginosa*, 8 (6%). Based on the results obtained in this study, the diameters of the inhibition zone for bacterial isolates were increasing as the volume of added oil increased. When adding 10 µl of sesame oil the inhibition zone diameters ranged (8–10 mm), when adding 15 µl inhibition zone diameters ranged (14–18 mm), and when adding 20 µl the inhibition zone diameters ranged (20–26 mm), [Table 1].

In the second method, the inhibition zone diameters ranged at a concentration of 10% (10–14 mm) and at a concentration of 20%, inhibition zone diameters ranged (18–22 mm), while the concentration of 30% inhibition zone diameters ranged (22–28 mm), [Table 2].

As can be seen, there is a meaningful relationship between increasing the volume and concentration of sesame oil and reducing the growth of bacteria, so that by increasing the volume and concentration, a significant reduction in bacterial growth can be detected. In other words, the synergy of sesame oil and antibiotics has shown to have an effective effect on CIP-resistant Enterobacteriaceae isolated from urinary tract infection and diarrhea. A study that evaluated the antimicrobial property of sesame radiatum oil on the same bacteria to our study with the agar disk diffusion method measured the zone of inhibition 10–19 mm.[16] This implies the effectiveness of the oil in inhibition of the growth of bacteria.

**Table 1: The diameter of the inhibition zone as in agar diffusion method**

| Species | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | The volume of oil added with the antibiotic disc | | | |
| | CIP | CIP+10 µl | CIP+15 µl | CIP+20 µl |
| *Escherichia coli* | NZ | 8 | 16 | 22 |
| *Klebseilla* | NZ | 10 | 18 | 26 |
| *Salmonella* | NZ | 10 | 14 | 22 |
| *Proteus* | NZ | 8 | 14 | 20 |

NZ: No zone inhibition, CIP: Ciprofloxacin

**Table 2: The diameter of the inhibition zone in method of ensure the solubility of oil in the microbial medium**

| Species | Zone of inhibition (mm) | | | |
|---|---|---|---|---|
| | DMSO | The concentration of sesame oil in nutrient broth | | |
| *Escherichia coli* | NZ | 12 | 18 | 24 |
| *Klebsiella* | NZ | 14 | 22 | 28 |
| *Salmonella* | NZ | 10 | 18 | 24 |
| *Proteus* | NZ | 10 | 18 | 22 |

NZ: No zone inhibition, DMSO: Dimethyl sulfoxide

In another study on the bactericidal properties of Lignans in sesame seeds on three bacterial species, the obtained results showed complete inhibition of the growth of *Bacillus cereus* when facing the sesamol in the concentration of 2 mg/ml.[17] A study on the synergism of sesame oil and canola oil showed that the mixture of these two oils is more antioxidant than each oil individually. In another study, the mixture of the sesame oil and olive oil not only was not more antibacterial than each oil but also it had a less antibacterial effect than olive oil.[18,19]

According to previous studies, sesame oil shows different therapeutic characters, which is consistent with the current study, which indicates its antibacterial properties on Enterobacteriaceae.[3] It can be said that the use of this extract will be a natural substance that provides the body with important compounds as well as the inhibitory effect of pathogenic *Klebsiella* bacteria when used by patients and this was the main purpose of using this extract against bacteria causing urinary tract infection.[20] However, the most important point in this study was focused on the synergistic characters of sesame oil and CIP, which showed clear results by measuring the diameter of the zone inhibition, however, did not have the effect of sesame oil or antibiotic in inhibiting the growth of bacteria when added severally.

## CONCLUSION

The results of this study showed that there is an important effect of sesame oil synergistically with CIP on the Enterobacteriaceae resistant to this antibiotic and causing urinary tract infection and diarrhea and did not show the same effect when adding sesame oil or antibiotics separately.

## REFERENCES


1. Sefidgar A, Taghizadeh AM, Pournajaf A, Ardebili A, Omidi S, Abdian AA. Evaluation of antimicrobial activity of alcoholic and aqueous extracts from common hop (*Humulus lupulus*) and oak (*Quercus castaneifolia*). Evaluation 2015;17:39-46.
2. Sadrnia M, Arjomandzadegan M. Comparative study on the effects of *Aloe vera* extract in clinical strains of *Staphylococcus aureus*, *Klebsiella*, *Staphylococcus epidermidis* and *Escherichia coli* compared to antibiotics of choice. Arak Med Univ J 2014;17:39-46.
3. Heidari-Soureshjani R, Abolfazl G, Zia O, Arman J, Safiyeh A, Yaghoub M. Bactericidal and bacteriostatic effect of sesame oil, olive oil and their synergism on *Escherichia coli in vitro*. Adv Herb Med 2016;2:7-12.
4. Andersen T, Khandige S, Madelung M, Brewer J, Kolmos H, Møller-Jensen J. *Escherichia coli* uropathogenesis *in vitro*: In vasion, cellular escape, and secondary infection analyzed in a human bladder cell infection model. Infect Immun 2012;80:1858-67.
5. Boniadian M, Habibian R, Barati S, Jostejo T. Investigation the antibioticresistance of the *Escherichia coli* isolated from gastroenteritis cases in Shahrekord County. J Affil Shahrekord Univ Med Sci 2013;15:117-23.
6. Abdollahi KS, Najafipour S, Kafilzadeh F, Abdollahi A, Jafari S, Moravej A. Evaluation of drug resistance pattern of *Escherichia coli* strains isolated from FasaVali-e-Asr hospital patients. J Fasa Univ Med Sci 2013;2:273-8.
7. Malakootian M, Hatami B. Survey of chemical composition and antibacterial activity of *Rosmarinus officinalis* essential oils on *Escherichia coli* and its kinetic. Sci Inf Database 2013;12:1-13.
8. Zahra Z, Mojdeh HV, Fatimah BB. Contribution of GyrA and QnrA genes in ciprofloxacin resistant *Ecsherichia coli* isolates from patients with urinary tract infections of Imam Khomeini Hospital of Tehran. Arch Pediatr Inf Dis 2018;6:e62129.
9. Pourahmad Jaktaji R, Mohiti E. Study of mutations in the DNA gyrase gyrA gene of *Escherichia coli*. Iran J Pharm Res 2010;9:43-8.
10. Bagheri M, Molzemi S, Hadizadeh M, Aminian M. Study of alcohol extract of sesame on wound healing of diabetic male Wistar rats. J Neyshabur Univ Med Sci 2016;3:1-9.
11. Heidari-Soureshjani R, Obeidavi Z, Reisi-Vanani V, Ebrahimi DS, Fattahian N, Gholipour A. Evaluation of antibacterial effect of sesame oil, olive oil and their synergism on *Staphylococcus aureus in vitro*. Adv Herb Med 2016;2:13-9.
12. Shittu L, Bankole MA, Ahmed T, Bankole MN, Shittu RK, Saalu CL, *et al*. Antibacterial and antifungal activities of essential oils of crude extracts of *Sesamum radiatum* against some common pathogenic micro-organisms. Iran J Pharmacol Ther 2007;6:165-70.
13. Were BA, Onkware AO, Gudu S, Welander M, Carlsson AS. Seed oil content and fatty acid composition in East African sesame (*Sesamum indicum* L.) accessions evaluated over 3 years. Field Crops Res 2006;97:254-60.
14. Shittu L, Bankole M, Ahmed T, Aile K, Akinsanya M, Bankole M. Differential antimicrobial activity of the various crude leaves extracts of *Sesamum radiatum* against some common pathogenic micro-organisms. Sci Res Essay 2006;1:108-11.
15. Agah MJ, Nassiri-moghaddam H, Golian A, Raji AR,

Farhosh R, Zarban A. Effect of dietary olive leaves (*Olea europaea* L.) extracts and sesame (*Sesamum indicum* L.) oil on intestinal morphology and some blood parameters in broiler chicken Iranian. Vet J 2015;11:33-43.
16. Osibote E, Ogunlesi M, Okiei W, Asekun T, Familoni O. Assessment of antimicrobial activity of the essential oil from the stem powder of *Cissus populnea* and the leaves of *Sesamum radiatum*, herbal medications for male infertility factor. J Med Plants Res 2010;4:14-20.
17. Mahendra Kumar C, Singh SA. Bioactive lignans from sesame (*Sesamum indicum* L.): Evaluation of their antioxidant and antibacterial effects for food applications. J Food Sci Technol 2015;52:2934-41.
18. Mohammadi T, Hatami M, Mirzaee SY, Hooshiari A, Nejatian M. Formulation of blend oil containing canola and sesame oils without synthetic antioxidants. Iran J Nutr Sci Food Technol 2014;9:83-92.
19. Heidari-Soureshjani R, Reza A, Seyed SG, Reza HH, Arefeh K, Yaghoub M, *et al*. Study of the bactericidal and bacteriostatic effects of olive oil, sesame oil and their synergism on *Pseudomonas aeruginosa in vitro*. Adv Herb Med 2016;2:1-6.
20. Khan S, Kumar A, Adhikari JS, Rizvi MA, Chaudhury NK. Protective effect of sesamol against $^{60}$Co γ-ray-induced hematopoietic and gastrointestinal injury in C57BL/6 male mice. Free Radic Res 2015;49:1344-61.

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