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Evaluation of antimicrobial effects of *Zataria multiflora, Rosemarinus officinalis* and *Cuminum cyminum* essential oils on the growth of Escherichia coli O157:H7

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ABSTRACT

Due to the increased resistance to antibiotics, identifying new compounds with fewer side effects is more important than usual medications in treatment of microorganisms. This study aimed to examine the antibacterial activity of *Zataria multiflora*, *Rosemarinus officinalis* and *Cuminum cyminum* essential oils on the growth of *E. coli* O157:H7 as one of the most common pathogens of food-borne diseases. *Z. multiflora*, *R. officinalis* and *C. cyminum* plants were dried and ground. Then the extraction of essential oil was performed by the hydrodistillation method. In order to examine the antibacterial effects, micro-broth dilution was used to determine the minimum inhibitory concentration (MIC) and minimum bactericidal concentrations (MBC). The disc diffusion method was applied to measure the inhibitory zone diameter. The data were analyzed by SPSS. Maximum diameter of inhibition zone (26.96 mm) was related to *Z. multiflora* which had a significant difference compared to other essential oils ($p<0.001$). Also the MIC and MBC of *Z. multiflora* essential oil were obtained as 3.125 mg/ml and 6.25 mg/ml, respectively. In this study, *Zataria multiflora, Rosemarinus officinalis* and *Cuminum cyminum* essential oils had significant antibacterial properties against *E.coli* O157:H7. As a result of these essential oils after completing studies, they can be a good alternative to chemical preservatives for storage of food.

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1. Introduction

Over the past decades, food borne diseases are considered as one of the leading public health threats worldwide. These diseases are classified as intestinal diseases and have been increasing, not only in developing countries, but also in developed countries (1,3). Various factors contribute to the transmission of food-borne diseases including bacteria, viruses, parasites, fungi and some toxins (4,5) that bacteria and toxins derived from them are the most common cause of food-borne diseases (6,7).

E. coli O157:H7 is one of the most important serotypes of enterohemorrhagic Escherichia coli (EHEC) that due to low infectious dose (less than 100 pcs), these bacteria are transmitted to human by consumption of contaminated food (8,9). STX1 and STX2 are the most important toxins in these bacteria that with other bacteria cause some disease such as hemolytic uremic syndrome (HUS), hemorrhagic colitic and thrombotic thrombocytopenic purpura (12,16).

Studies have shown that various extracts and essential oils prevent the growth of a wide range of pathogenic microorganisms. A reason for this approach can be seen in the presence of natural origin and the lack of adverse effects in humans and in the environment as to chemical preservatives (17).

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Examples of medicinal herbs with their antimicrobial effects are Z. multiflora, R. officinalis, and C. cyminum. Z. multiflora is the most well-known medicinal herb which grows in Iran, Afghanistan and Pakistan. This herb is known as an antiseptic, anti-spasm and anti-inflammatory agent in traditional medicine. Also, Z. multiflora is widely used as a food flavoring that is related to phenolic compounds such as caracrole, eugenol and thymol in this herb (18,19).

R. officinalis, commonly known as rosemary, is widely known as a spice and used in traditional medicine (20,21). It is a member of the mint family Lamiaceae and is native to Asia and some other parts of the world. Also, *C. cyminum* can be used in the treatment of digestive and pulmonary diseases (22,23).

Considering a wide range of medicinal herbs in Iran, the present study was conducted to examine the effect of the concentrated essential oils of *Z. multiflora, R. officinalis and C. cyminum* on the growth rate of *E.coli* O157: H7.

2. Materials and methods

2.1. Preparation of essential oil

For essential oil preparation, plants of *Z. multiflora*, *R. officinalis* and *C. cyminum* were obtained in spring of 2016 from Maragheh and Tabriz cities, Iran. For the identification, these plants were sent to Department of Plant Biology of Payame Noor University of Maragheh. The plants were dried in shading conditions and 50 g of them were individually powdered by electric grinders and added to a balloon containing 700 ml of distilled water and were prepared for Hydrodistillation. The essential oils were dewatered and dried using Sodium Sulfate and Whatman Filter (Whatman 2) and kept in dark-colored containers away from light and kept in a fridge (24).

2.2. Bacterial strains

For evaluation of the antibacterial effect of essential oil, the standard strain of *E. coli* O157: H7 NCTC12900 was selected. For fresh culturing of isolates, nutrient broth medium (Merck, Germany) was used and to provide a uniform bacterial suspension, after 24 hours of culturing of strains, 2-3 colonies were inoculated into 5 ml sterile normal saline and placed at 37°C for 30 minutes until the opacity was equivalent to 0.5 McFarland standard (1.5×10^8 cfu/ml) (25).

2.3. Antibacterial activity of the essential oil

Antimicrobial properties of the essential oils were determined using the disc diffusion method and based on Clinical and Laboratory Standards Institute Protocols (CLSI) (26). The essential oils were solved in dimethyl sulfoxide (DMSO) solvent and after preparation, concentrations of 200, 100, 50, 25, 12.5, 6.25, 3.125 and 1.562 mg/ml were sterilized by passing through a 0.22 µm pore size filter. After culturing of bacterial strain on Mueller-Hinton agar plates, 6 mm paper discs containing the essential oils with concentrations of 10 ul were placed on plate surface and were incubated at 37°C for 18-24 hours. Enrofloxacin (5 µg), flumequin (30 µg), oxytracycline (30 µg) and erythromycin (15 µg), were used for antibiotic susceptibility testing and discs containing DMSO were used as negative control. All experiments were performed three times (25).

2.4. Minimum Inhibitory Concentration (MIC)

Micro-broth dilution method was used to determine the minimum inhibitory concentration, according to the CLSI protocol (26). Briefly, 20 µl of the essential oils dissolved in DMSO was added to each 96-well microplate well containing 160 µl Mueller-Hinton broth (Merck, Germany) and serial dilutions were provided. Then, 20 µl of microbial suspension containing 10^6 cfu/ml was added to each well of the microplates. The negative and positive controls of the culture medium and bacterial suspension were added to the wells. Then all microplates were incubated at 37°C for 18-24 h.

2.5. Minimum Bactericidal Concentration (MBC)

For this section, 5 µl suspension from all wells without turbidity in the MIC phase were cultured on the Mueller-Hinton agar medium. Afterwards, the plates were incubated at 37°C for 24 h. The lowest concentration of essential oil in which the bacteria were killed was regarded as MBC. The mean values of inhibition zones were compared using one-way ANOVA and SPSS 23.

3. Results

The results of this study revealed that essential oils of *Z. multiflora*, *R. officinalis* and *C. cyminum* can affect the growth of *E.coli* O157:H7. The lowest inhibitory levels of growth were observed at 10 mg/ml of *Z. multiflora* and *R. officinalis*. *Z. multiflora* had the highest effect on *E.coli* O157: H7 (with 26.96 mm diameter of the inhibition zones) that in this regard, a significant difference was observed between the other essential oils (p<0.001). Also, *Z. multiflora* with MIC= 3.125 and MBC=6.25 mg/ml had an antimicrobial activity equivalent to tetracycline and much more than erythromycin. The results of antimicrobial activity of essential oils and antibiotics are shown in tables 1 and 2.

Table 1. The mean diameter of the inhibition zones in the standard strain of *E.coli* O157: H7 (the concentration of 10 mg/ ml)

Essential oil	The average diameter of the inhibition zones (mm)
Zataria multiflora	26.96
Rosemarinus officinalis	14
Cuminum cyminum	-

Table 2. The MIC and MBC of antibiotics and essential oils against the standard strain of *E.coli* O157: H7

Antibiotic	(MIC) (mg/ml)	(MBC) (mg/ml)
Enrofloxacin	0.781	1.562
Flumequin	1.562	3.125
Oxytracycline	3.125	6.25
Erythromycin	6.25	12.5
Zataria multiflora	3.125	6.25
Rosemarinus officinalis	12.5	25
Cuminum cyminum	25	50

4. Discussion

The findings of this study showed that *Z. multiflora, R. officinalis* and *C. cyminum* essential oil have antimicrobial effect on *E.coli* O157:H7. These bacteria are one of the most important pathogenic agents of gastrointestinal diseases that can lead to high mortality during epidemics and outbreaks due to the consumption of contaminated foods (13). Hence, one of the main concerns of food industry owners and consumers of food is contamination with *E.coli* O157:H7 and other microbial agents. In many cases, antibiotics and chemical preservatives are used to boost the shelf-life of foods and block the pathogenic bacteria's growth or reproduction. The growth of bacteria can lead to food corrosion that causes a lot of public health problems. One of the problems is increasing of resistance to antibiotics in food-borne bacteria. Therefore, one of methods for controlling of food pathogens is the use of the plant-derived compounds (28,29).

In recent years, pharmaceutical companies have carried out extensive research on the discovery of safe and effective antimicrobial agents for the prevention and treatment of a wide range of multi-drug resistance bacterial infections. Because of antimicrobial effects of the essential oils and their lower side effects, these agents can be used as an appropriate alternative to antibiotics (30). The results of this study evinced that the essential oils of *Z. multiflora* and *R. officinalis* can inhibit the growth of *E. coli* O157: H7. As the concentration of the extracts increased, the inhibition zone increased and the highest inhibitory effect of the bacteria was observed at a concentration of 10 mg/ml. Also, the essential oil of *Z. multiflora* was significantly more effective than other essential oils (p<0.001) and its antimicrobial effect was much stronger than erythromycin. In recent years, the antimicrobial effects of the extracts and essential oils have been reported (31).

Soltan Dallal et al. studied the effect of *Z. multiflora* on antibiotic resistant Staphylococcus aureus strains isolated from the food. They reported that these agents significantly can reduce the growth of *S. aureus* (34). Also, Yaghobzadeh et al. in 2011 investigated the effect of *Z. multiflora* on shiga-toxin producing E. coli strains. In this study, the mean diameter of the bacterial inhibition zone was 28.18 mm, which is in line with the findings of the current study (35). In relation to the antibacterial effects of R. officinalis, Soltan Dallal et al. showed that the diameter of the inhibition zone was observed about 20 mm in methicillin-resistant staphylococci isolated from patients and foodstuffs (30). In another study by Malakootian and Hatami, the diameter of the inhibition zone was 16±1.07 mm, which is similar to the findings of the current study. It was also found that the MIC from the growth of the E. coli was 3000 µg/ml and its MBC was 3200 µg/ml (25).

Also, studies have done on the antimicrobial effect of *C. cyminum* (23,36,37,38). According to the findings of the present study, the essential oil of *C. cyminum* had a very low inhibitory effect and less than <1 mg/ml on the *E. coli* O157: H7. This could be due to the concentration of essential oils tested in this study, because Suliman in Iraq (2009) showed that 400 mg/ml of methanolic extract of *C. cyminum* have inhibitory effect on the growth of a number of Gram-negative bacteria (39). Similarly to the results of this study, Haghighi et al. reported that *C. cyminum* in comparison with other essential oils had the lowest antifungal effect on *Candida albicans* (40).

Due to the many antimicrobial effects of the essential oils, they can be used as natural preservatives to increase the shelf-life of foods. The limitations of the present study are not to use concentrations higher than 10 mg/ml because the use of high concentrations of extracts and essential oils, in some cases, causes changes in the organoleptic properties of foods (31). According to the results of this study, it is possible to develop appropriate drugs for the elimination of food-borne bacteria by using these essential oils.

5. Conclusion

This study showed that essential oil of *Z. multiflora* and *R. officinalis* have antimicrobial effects against *E. coli* O157: H7, which can be used as an appropriate herbal source against microorganisms and treatment of human bacterial pathogens.

Conflict of interest

The authors declared that they have no conflict of interest.

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