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Original research

Antimicrobial effect of clove alcoholic extract and essential oil on *Escherichia coli* O157: H7

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ABSTRACT

The importance of using different medicinal plants indicates their health and valuable effects such as antioxidant, antimicrobial, anti-inflammatory effects and etc. In this research, the effect of alcoholic extract and essential oil of *Syzygium aromaticum* on *Escherichia coli* was investigated. The effect of the plant on this microorganism as a gram negative was evaluated and important indicator of poisoning and diarrhea at levels of 3, 4, 5, 6, 7, 8 and 9 mg/ml of broth culture media. By dilution method using liquid culture media, minimum growth inhibitory concentration and minimum bactericidal concentration were determined. The results of this study indicated increasing the concentration of extract and essential oil in the studied area increased their antibacterial effect and the most inhibitory effect was on essential oil which means that the minimum inhibitory concentration for ethanolic extract and essential oil was 8 and 6 mg/ml, respectively. Similar results were observed in relation to MBC. Also, by comparing the obtained results, it was deduced that the inhibitory effect of the essential oil used in this study on *E. coli* was greater than the clove extract and the essential oil of the cloves had a more antibacterial role than extract. Eugenol, eugenol acetate, cavicol and beta-caryophyllene were observed in GC-MS analysis and the difference in the content of bioactive ingredient eugenol further confirmed this antimicrobial effect.

Keywords: Essential oil; *E. coli*; Alcoholic extract; *Syzygium aromaticum*; GC-MS

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1. Introduction

In the present century, maintaining the safety of food and its quality during the shelf life is something that not only attracts the attention of food industry experts and health officials, but lack of attention to it can cause irreparable damage to society. Today, diseases caused by the consumption of contaminated food are a major problem all over the world, even in developed countries such as the United States. *Escherichia coli* O157: H7 is the cause of several infections and deaths in the world in recent decades. This strain of *Escherichia coli* was first identified in 1982 in two cases of foodborne infections in the United State (Burt, 2004). This bacterium is in many cases similar to *Shigella* and can produce a toxin similar to Shigatoxin. The pathogenesis of this *Escherichia coli* is colon and the disease can be asymptomatic, normal diarrhea, hemorrhagic diarrhea or hemolytic colitis and etc. The first recorded case of *Escherichia coli* O157: H7 infection was in 1982 in Oregon, USA, where 26 people were hospitalized after consuming undercooked hamburgers in a chain restaurant, and 19 of them were hospitalized with heartburn and dysentery. In studies, this type of *Escherichia coli* was isolated from patients' feces as well as frozen hamburgers (Burt, 2004). Extracts and essential oils are produced and supplied almost from the thirteenth century, with the development of various branches of science, the use of chemicals in the production of the drug, the researchers' attention was focused on the indiscriminate. The use of antibiotics leads to resistance drug against different antibiotics has been in human. This issue at motivating researchers using herbs as natural products safe, accessible and inexpensive compared to synthetic antibiotics used to treat bacterial cause (Misaghi & Akhondzadeh basti, 2007; Shariat, 1998).

The first measurement was made in 1881 to determine the antibacterial properties of extracts (Shan et al., 2007; Jabary khamen et al., 2008). The use of plant extracts and essential oils as an alternative to synthetic preservatives has found its place in the food industry (Sonboli et al., 2007; Abdelwahed et al., 2006; Kukcuoglu et al., 2006). Plant essential oils are aromatic oily

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liquids obtained from various parts of plants and used as food flavorings (Burt, 2004). In this regard, studies have shown that clove is an important plant source that has antibacterial, antifungal, antiviral properties and includes phenolic compounds such as flavonoids, hydroxy benzoic acid, hydroxy cinnamic acid and propene hydroxy phenyl and essential oils such as eugenol, carvacrol, thymol, and menthol (khoshdouni farahani & khoshdouni farahani, 2017). Hoque et al. (2008) investigated the antimicrobial activity of clove and cinnamon extracts against foodborne pathogens and spoilage bacteria. The results showed that clove essential oil can be useful for controlling them. Farhangfar et al. (2011) in a study investigated the effects of different concentrations of clove essential oil and grape seed extract alone and in combination. The results showed that despite the significant antimicrobial effects of clove essential oil in controlling spoilage compared to the control group, grape seed extract in the concentrations used did not have good antimicrobial effects in controlling microbial spoilage. Hiwandika et al. (2021) was investigated antibacterial and antifungal activity of clove and the results showed that the main constituents of clove extract are eugenol and β-caryophyllene, which are antibacterial and antifungal agents. Clove ethanolic extract has the ability to inhibit gram-positive and gram-negative bacteria such as *S. aureus*, *B. cereus*, *E. coli*, *P. aeruginosa*, etc. In another research, antimicrobial activity of some essential oils (tea tree oil, oregano oil, clove oil, cinnamon oil, rosemary oil, black pepper oil, etc.) from aromatic plants on *Staphylococcus aureus*, *Escherichia coli* strain and *Candida albicans* yeast was studied (Rota et al., 2004). The results depicted that *S. aureus* strains were very sensitive to different types of essential oils and *E. coli* was favorably affected by these EOs. Due to the antibacterial potency of extracts and essential oils and their effectiveness against microorganisms, the aim of this study was to investigate and compare the antibacterial effect of alcoholic extract and clove essential oil simultaneously. It is also a comparison of the amount of effective compounds in the extract and essential oil that are also effective on the antibacterial effect.

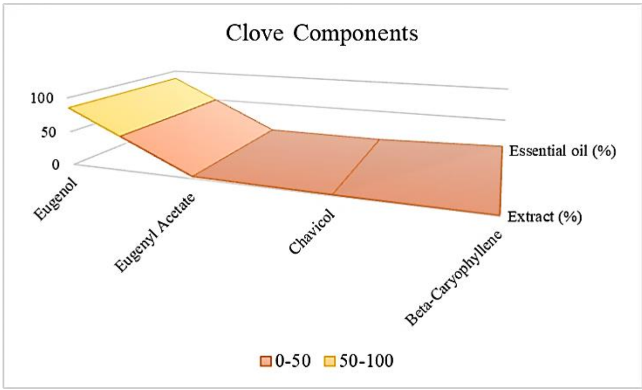

Fig. 1. Major components of clove essential oil and extract.

2. Material and Methods

2.1. Preparation of clove plant

Clove plant was purchased from the Tehran grand bazaar of medicinal plants in Tehran and the plant was delivered to the university center and its characteristics were determined by number BASU 23709 and species *D. orientalis*. After sunlight drying the plant, they were transferred to laboratory.

2.2. Extraction of clove extract

Immersion method was used to extract the dried clove. Clove powder with 70% ethanol was mixed in an erlenmeyer with a dark lid, and the sample was shaked (150 rpm) for 48 h, after which the ethanolic extract was obtained by passing the mixture through filter paper. Then the extract was concentrated using a rotary evaporator under vacuum and the solvent was separated (Taherkhani et al., 2016; Khoshdouni Farahani & Khoshdouni Farahani, 2017).

2.3. Preparation of essential oil

Celevenger method was used for essential oil extraction, which was performed based on the method of Taherkhani et al. (2015). Also, a 0.45 micron head filter was used to sterilize the clove extract and essential oil.

2.4. Analysis of volatile compounds of clove extract and essential oil

Identification and analysis of extract and essential oil compounds were performed by gas chromatography with mass spectrometer (MS/GC) (Hewllet Pachard, 6890-H model, USA) with the following specifications: column type (methyl silicon-cross link) HP-1MS, column dimensions; 60 m long, 0.20 mm diameter, initial and final temperature: 162 and 132 ^{o}C and injection site temperature 152 ^{o}C (Khoshdouni Farahani & Khoshdouni Farahani, 2017).

2.5. Antimicrobial analysis

3, 4, 5, 6, 7, 8 and 9 mg/ml of extract and essential oil were prepared to compare their identical concentrations, which were selected based on previous studies and preliminary tests selected in this study. For this purpose, *Escherichia coli* strain O157: H7 was provided by a microbiology laboratory in science and research branch of Islamic Azad University.

2.6. Dilution method

By dilution method using broth culture medium, minimum growth inhibitory concentration and minimum bactericidal concentration were determined. 9 ml of liquid Müller-Hinton culture medium with clove extract and essential oil of a certain concentration along with 1 ml of the microorganism culture suspension to the test tubes were added. The half-McFarland standard (The adsorption of half-McFarland solution at 625 nm is between 0.08 and 0.13) creates a turbidity equivalent to a bacterial suspension containing 1.5×10^8 cfu/ml that was used. The essential oil of the plant was dissolved in 10% dimethyl sulfoxide solution. The tubes were then incubated at 35 °C for 24 h. Tubes in which bacteria did not grow and did not have turbidity, a tube containing extract and essential oil as MIC were reported. To determine the minimum bactericidal concentration (MBC), one ml of tubes in which no germ had grown was mixed with 15 ml of Müller-Hinton agar melt at 48 °C in a plate (pour plate as a culture method) and then was incubated at 35 °C for 48 h. Then the plate containing the lowest concentration of extract and essential oil in which no growth was observed was considered as MBC of extract and essential oil (Vanden et al., 1991).

2.7. Statistical analysis

In this study, a completely randomized design was used to analyze the data and duncan's multiple range test using 26 SPSS software (SPSS Inc., Chicago, USA) was used to compare the means obtained.

3. Results and Discussion

3.1. GC-MS analysis

The results of systemic analysis showed the highest content of compounds that were present in both essential oil and clove extract, included eugenol, eugenol acetate, cavicol and beta-caryophyllene, respectively (Fig. 1). The functional properties are related to the maximum content of eugenol, which is higher in the essential oil (86.04%) rather than extract (85.2%) and its effect on microbial analysis is expected to be clearly seen. The results of Khoshdouni Farahani and Khoshdouni Farahani (2017) also confirm these data.

3.2. Antimicrobial analysis

The results of dilution broth test showed that clove extract in liquid medium had antibacterial effect on *Escherichia coli* at the highest concentrations (8-9 mg/ml) used and its essential oil had antibacterial effect in the final concentrations, as well. At lower concentrations (3-6 mg/ml) no growth was observed in them, this lack of growth is shown negatively in Table 1 and positive growth is shown if bacterial growth is observed. The ethanolic extract had a MIC of 8 mg/ml and the essential oil of the plant had a MIC of 6 mg/ml. Similar results were obtained with respect to the minimum bactriocidal concentration. The MBC levels of ethanolic extract and plant essential oil were 9 mg/ml and 7 mg/ml, respectively (Table 2).

Plant-derived natural compounds are considered as synthetic substituted antimicrobial compounds and they have fewer side effects. In our country, the importance of research and study on medicinal plants has been identified (Mousavi, 1981). Several factors affect the antimicrobial properties of a plant. Factors such as the content of essential oil of the plant, the method of extraction and the type of solvent used and the concentration of the extract can affect the antimicrobial properties (Singh et al., 2003; Cosentino et al., 1999; Rasodi et al., 2006).

This study showed that clove essential oil has antimicrobial effects on *Escherichia coli*. This finding is consistent with the results of Hosseini et al. (2015) study. The results of this study showed that clove essential oil has a stronger inhibitory effect on *Escherichia coli* with MIC = 6 mg/ml compared to the alcoholic extract of this plant. In a study conducted by Noori et al. (2011) on the effect of preserving cinnamon essential oil on the growth rate of *Escherichia coli* O157:H7 in hamburgers using a combination technology, similar results were obtained. This means that with increasing the concentration of essential oil, the growth rate of bacteria decreased.

Another factor that may affect the antimicrobial effects of a plant extract is the method of extraction and the type of solvent used. Extracts extracted from a plant by different methods and solvents can have different antimicrobial effects on specific species of microorganisms (Nostro et al., 2000). Ibrahim and Osman (1995) showed that ethanol extract of *Cassia Alata* leaves has antifungal effect against four fungal species but is ineffective on yeasts and bacterial species, but the antibacterial effects of methanolic extract of this plant has been proven by Khan et al. (2001). In the present study, different effects of extract and essential oil were observed. Unlike clove extract, clove essential oil had a greater effect on *Escherichia coli*.

Since more antimicrobial effects were observed in essential oil compared to ethanolic extract, it can be concluded that the bioactive compound of clove was more in essential oil, which was also confirmed by the results of gas chromatography. Other researchers have noted the susceptibility of different bacterial species to antimicrobials (Penna et al., 2001). The mechanism of action of clove is described in such a way that the essential oil and clove extract have antibacterial, antifungal and antioxidant properties. The bioactive compound in clove is eugenol. High levels of this compound in clove essential oil and extract cause its biological activity and strong antibacterial properties. This phenolic compound denatures proteins and reacts with cell wall phospholipids. Thus, it changes the permeability of the cell wall (Wenqiang et al., 2007). In a study was done by Matan et al. (2006), the addition of 4,000 microliters of clove essential oil inhibited the growth of *Aspergillus flavus*. Also, Valero & Salmeron (2003) observed that clove essential oil reduced the growth of *Bacillus cereus*. On the other hand, Benchaar et al. (2007) showed in a study that phenolic compounds, including eugenol, the active ingredient in cloves, have antimicrobial activity. This effect is due to the presence of hydroxyl groups in their phenolic structure. Phenolic compounds have a wide range of antimicrobial activity against gram-positive and gram-negative bacteria (Lambert et al., 2001).

Table 1. Minimum concentration of inhibitor (MIC) of clove ethanol extract and essential oil against *Escherichia coli* microorganisms, concentrations in terms (mg/ml).

	Positive Control**	Negative Control*	3	4	5	6	7	8	9
Ethanol extract	+	-	+	+	+	+	+	+	-
Essential oil	+	-	+	+	+	+	-	-	-

(-): Observation of lack of growth of microorganism.
(+): Observation of growth of microorganisms.
*Culture medium + extract or essential oil.
**Culture medium + Microbial suspension.

Table 2. MBC of clove ethanol extract and essential oil against *Escherichia coli* microorganisms, concentrations in terms (mg/ml).

	Positive Control**	Negative Control*	7	8	9
Ethanol extract	-	-	-	-	-
Essential oil	-	-	-	-	-

(-): Observation of lack of growth of microorganism.
*Culture medium + extract or essential oil.
**Culture medium + Microbial suspension.

Microbial activity of clove essential oil (*Eugenia caryophyllata*) was investigated by Nuñez & D'Aquino (2012). The results showed that *Escherichia coli* was highly sensitive to clove essential oil and could be considered as a potential antimicrobial agent. Antibacterial, antifungal and antioxidant activity of cinnamon and clove essential oils was studied (Purkait et al., 2020). The combination of cinnamon and clove essential oil shows synergistic antibacterial activity against food-borne bacteria including *Staphylococcus aureus*, *Listeria monocytogenes*, *Salmonella typhimurium* and *Pseudomonas aeruginosa*, and synergistic antifungal activity against *Aspergillus niger*. The results show evidence that the combination of cinnamon and clove essential oil can be used as a potential source of antibacterial, antifungal and natural antioxidant compounds in the food and pharmaceutical industries. Antibacterial activities of cinnamon, white thyme, and clove essential oils were investigated (Valdivieso-Ugarte et al., 2021). The results showed antipathogenic microbial activity of cinnamon, thyme and clove, respectively. Effect of clove (*Syzygium aromaticum*) on resistant bacteria and Organoleptic Quality of meat was surveyed (Tshabalala et al., 2021). The outcomes show that clove and black seed cumin extract have excellent antibacterial activity against most pathogenic bacteria. Clove shows the highest inhibition zone against *E. coli*. Clove extract was the most inhibitory followed by black cumin, while thyme and cinnamon extracts showed weak antibacterial activity against the tested strains. All of these studies confirm the effectiveness of clove antimicrobial compounds on bacteria. The results showed a stronger antibacterial action of clove essential oil with MIC equal to 7 mg/ml compared to ethanol. The antimicrobial effects of this essential oil can be attributed to the compounds of eugenol, eugenol acetate and beta-caryophyllene, which have the highest percentage of chemical compounds.

4. Conclusion

In this study, the antimicrobial effect of clove extract and essential oil as a plant with antimicrobial potential on *Escherichia coli* O157: H7 was investigated. Their microbial properties were investigated using broth dilution method and MIC and MBC analyzes were performed. The outcomes showed that the essential oil was more effective than the extract due to the presence of more bioactive compounds in the essential oil. Also, different concentrations of extract and essential oil are effective in the effect of antimicrobial properties and in several studies with changing the concentration of extract and essential oil, the antimicrobial effects of the plant have changed. In some cases, clove plant was studied as a plant with many potential properties. GC instrumental analysis indicates the effect of different content of bioactive ingredients on plant performance characteristics. Due to the higher concentration of the active ingredient in plant residues, it is very likely that the study of antimicrobial effects will provide a wider range of information for operational applications.

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Conflict of interest

The authors declare that they have no known competing financial interests.

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