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# Investigating the effect of garlic (Allium sativum) essential oil on foodborne pathogenic microorganisms

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**Abstract**

Garlic is a plant that is native to Central Asia and belongs to the Alliaceae family. When garlic bulbs are crushed, they release a sulfoxide molecule called alliin, which is the precursor to the garlic's distinctive scent and flavor. Fresh garlic and its essential oil are used in the food sector as natural antioxidant agents, flavorings, and antimicrobials, notably in processed chicken and meat products. In this research, on a number of pathogenic strains, the garlic essential oil's antibacterial activity was investigated *in vitro* using minimum bactericidal concentration, minimum inhibitory concentration, and the disk diffusion technique. Gas chromatography was used to identify the garlic essential oil's chemical components. Aluminum chloride colorimetric, Folin-Ciocalteu, and radical reduction capacity methods were used to measure flavonoid, total phenol, and antioxidant content. It was shown that the most resistant to essential garlic oil were gram-negative bacteria, including Escherichia coli and Pseudomonas aeruginosa. The mixture of diallyl disulfide was 40.3% greater, according to the results of the chemical compound identification of essential garlic oil. Antioxidant activity, flavonoids, and total phenol of essential garlic oil were 80%, 0.24 mg quercetin in grams, and 0.33 mg gallic acid in gram, respectively. The findings of this investigation revealed that garlic might be exploited as a source of medicinal chemicals.

**Keywords:** inhibition zone diameter; reducing radical capacity; total phenol; garlic essential oil.

**Practical Application:** In the current study it was tried to investigate the garlic essential oil's antibacterial activity on a number of pathogenic strains.

## 1 Introduction

Drug-resistant bacteria that have developed novel resistance mechanisms, leading to antimicrobial resistance, continue to pose a danger to our capacity to treat common diseases (Soloviy et al., 2020). Over the last few decades, dangerous antibiotic-resistant microorganisms have become increasingly common (Perales-Adán et al., 2018; Pérez-Rodríguez & Taban, 2019; Younis et al., 2019). Because of the rise in infectious diseases and pathogenic microorganisms' resistance to chemical drugs over time, as well as the side effects and high treatment costs that chemical and synthetic drugs impose on human societies, the use of medicinal plants of natural origin has become more common in recent years (Subramani et al., 2017; Mulat et al., 2019; Gorlenko et al., 2020). Diseases transferred to humans through food have become a big problem in recent years, and the negative side effects of synthetic preservatives added to food have led to skepticism regarding these preservatives (Almeida et al., 2018; Rohr et al., 2019). As a result, several researchers have been driven to do follow-up studies to replace these preservatives with herbal and natural substances that produce similar results while having fewer side effects (Subramani et al., 2017; Helal et al., 2019; Zhao et al., 2019).

Essential oils and plant extracts, which are volatile and aromatic chemicals with good antibacterial activity, are among these components (Cui et al., 2020; Shin et al., 2022). Garlic, scientifically known as Allium sativum, is a member of the Amaryllidaceae family. This plant is employed to cure infectious diseases in traditional medicine (Ayaz & Alpsoy, 2007; Goncagul & Ayaz, 2010; Daka, 2011). This plant was used to treat a variety of ailments in ancient texts and literature, including heart disease,

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high blood cholesterol, arthritis, fever, cough, and headache. Garlic contains sulfur compounds, the most important component of which is called allicin. This compound affects pathogenic microorganisms and causes the treatment of diseases (Ankri & Mirelman, 1999; Rahman, 2007). Various studies show that there is a group in allicin called thiosulfinate, which binds to a protein and destroys microorganisms. El-Azzouny et al. (2018) showed essential garlic oil has a stronger antibacterial impact against gram-positive bacteria. The rod-shaped, gram-negative bacterium Escherichia coli belongs to the Enterobacteriaceae family. Most strains of this bacterium cause infections such as urinary tract infections, meningitis and peritonitis (Gould, 2011; Jang et al., 2017).

Pseudomonas aeruginosa is a pathogenic and gram-negative bacterium that inflicts diseases such as wound infections, pneumonia, gastric and respiratory infections that can lead to death if left untreated (Aoki et al., 2018; Cillóniz et al., 2019). Staphylococcus aureus is a gram-positive and pathogenic bacterium. Because this bacterium has become resistant to antibiotics over time, it is important to discover new ways to fight it (Banerjee & Ray, 2017; Liu et al., 2018; Yusof et al., 2019). The bacterium Listeria monocytogenes is gram-positive. It can tolerate up to 10% salt and drought, and water and food sources are the best places for this bacterium to grow and multiply (Wu et al., 2017; Sauer et al., 2019). This bacterium can cause dangerous diseases such as septicemia, encephalitis, and miscarriage. In the world, only a few studies on the identification of chemical compounds and the antimicrobial effect of essential garlic oil have been conducted (Osorio et al., 2021; Rezende et al., 2020). The majority of studies have focused on the antimicrobial effects of garlic extract, with very little research on the antimicrobial effects of garlic essential oil (Olugbue et al., 2017; Strika et al., 2017; Fufa, 2019; Uçak, 2019).

The purpose of this research was to figure out what chemicals components are in garlic essential oil by exploiting a gas chromatography device connected to a mass spectrometer (GC-MS), as well as determination of antioxidant activity, total phenol, flavonoid, and assessment of antibacterial activity of essential oil by an agar diffusion method called disk diffusion method, liquid dilution (minimum inhibitory concentration) and minimum bactericidal concentration on a number of pathogenic microorganisms (Staphylococcus aureus, Listeria innocua, and Pseudomonas aeruginosa) *in vitro*.

## 2 Material and methods

Dimethyl sulfoxide, tween 80, triphenyl tetrazolium chloride, gallic acid, 2,2-diphenyl-1-picrylhydrazyl, and alcohol 70 percent were among the chemicals used. A 96-well plate, Mueller Hinton agar, Mueller Hinton broth microbial media (Albano et al., 2020), and antibiotic discs of gentamicin and nalidixic acid were prepared. Essential oil extraction was performed using a Clevenger apparatus by steam distillation using 50 g of chopped garlic cloves and 600 mL of distilled water. The collection operation of essential oil lasted three hours. Finally, the essential garlic oil was collected by opening the mechanical valve.

### *2.1 Evaluation of the antimicrobial activity of garlic essential oil in vitro*

In this research, three antimicrobial methods have been used to assess the antimicrobial activity of essential garlic oil on a number of pathogens in vitro: minimum bactericidal concentration, minimum inhibitory concentration, and disk diffusion. A list of antimicrobial methods is provided below.

*Disk diffusion method*

First, Müller Hinton agar medium was prepared, autoclave sterilized, and transferred to Petri dishes in this method. 3 g of essential garlic oil was sterilized, and concentrations of 32.5, 75, 150, and 300 mg/mL were prepared using a syringe filter (diameter 0.22 µ). For 20 min, blank disks were soaked in various concentrations of essential garlic oil. 20 µL of microbial suspensions were taken and cultured on grass after being standardized with 0.5 McFarland solution. Using sterile forceps, two discs with different concentrations were placed at a distance from each other inside each petri dish and fixed in a suitable location (distance from the petri dish wall). The antibiotic disk nalidixic acid was used for the bacteria Escherichia coli and Pseudomonas aeruginosa, and the antibiotic disk gentamicin was used for the bacteria Staphylococcus aureus and Listeria innocua.

*Minimum inhibitory concentration*

The Behbahani & Fooladi (2018) methods were employed to discover the minimum inhibitory concentration (MIC). To summarize, different concentrations (0.5, 1, 2, 4, 16, 32, 64, 128, 256 mg/mL) of garlic essential oil were first prepared from a mother solution containing 512 mg/mL of garlic. 100 µL of the antimicrobial agent (essential garlic oil) and 10 µL of each pathogenic microbial strain (according to 0.5 McFarland standard) were poured into each of the 96-well microplates. After incubating the 96-well microplate for 24 h at 37 °C, ten microliters of 5% triphenyl tetrazolium chloride solution were added to each well, and incubation was repeated for 15 min. Garlic essential oil's minimum inhibitory concentration was established to be the first well in which no purple or pink discoloration was observed (Araújo et al., 2018; Knezevic et al., 2018).

*Minimum bactericidal concentration*

Using the results of the MIC test, a sampler was used to remove 100 µL of cells with no discoloration and culture them in Müller-Hinton agar medium. As per the previous tests, the warm-up procedure was carried out at a specific temperature and time. The minimum bactericidal concentration of essential garlic oil was reported as the first plate in which no colony was observed.

### *2.2 Identification of chemical compounds of essential garlic oil by gas chromatography*

Using gas chromatography (GC) and gas chromatography connected to a mass spectrometer (GC-MS), the chemical composition of essential garlic oil was determined. Briefly, 0.1 µL of essential garlic oil was injected into the GC/MS device.

The temperature of the column increased from 45 °C to 210 °C at a rate of 3 °C per minute. Garlic essential oil's components were identified by comparing them to standard mass spectra found in the literature (Jirovetz et al., 1992; Kimbaris et al., 2009; Dziri et al., 2014).

### *2.3 Determining the antioxidant activity of essential garlic oil*

To perform this test, the method of radical reduction was used. The antioxidant activity of extracts and essential oils is measured using this method. To conduct this test, three milliliters of essential garlic oil were mixed with one milliliter of DPPH (2,2-diphenyl-1-picrylhydrazyl) and left in the dark for 30 min. Finally, a spectrophotometer was used to determine the adsorption rate.

### *2.4 Total phenol*

Phenol was measured using the Folin-Ciocalteu method (Lamuela-Raventós, 2017). In this method, 1 mL of essential garlic oil was added to 2.5 mL of the Folin-Ciocalteu reagent diluted with water (1:10 ratio) and kept at room temperature (25 °C) for 2.5 min. After adding 2 mL of sodium carbonate, the solution was placed in a dark place for 1 h. Finally, the absorbance was measured at 725 nm.

### *2.5 Flavonoid compounds*

With minor modifications, the Zhishen et al. (1999) method was used to determine the flavonoid composition of essential garlic oil. 1 mL of essential garlic oil and 1.25 mL of distilled water were combined in this method. A 75-liter solution of sodium nitrite was added. Aluminum trichloride was added to the solution after 6 min and kept at room temperature for 5 min. Finally, 1 mL of sodium hydroxide was added its absorbance was measured at 510 nm.

### *2.6 Statistical analysis*

The tests were carried out three times, and the means were used for statistical analysis. Utilizing Duncan's Multiple Range Test (DMRT) and SPSS software at a significance level of $P < 0.05$, the data were analyzed.

## 3 Results and discussion

Garlic essential oil's antimicrobial activity against many food pathogens is influenced by its chemical composition, particularly the diallyl sulfide derivatives. Garlic cultivar and geographical origin have an impact on this essential element. The proportion of these diallyl sulfide derivatives in diverse cultivars from varied geographical origins, as well as the influence on antibacterial activity, have been observed to vary. Table 1 shows the antimicrobial activity of essential garlic oil on Pseudomonas aeruginosa, Escherichia coli, Staphylococcus aureus, and Listeria innocua using disk diffusion (Kirby-Bauer test).

The results of this test showed that the growth inhibition zone diameter observed around the disc for gram-positive bacteria (Listeria innocua and Staphylococcus aureus) was larger than that of gram-negative bacteria (Escherichia coli and Pseudomonas aeruginosa). The highest and lowest inhibitory zone diameters were observed in equal concentrations for Escherichia coli and Staphylococcus aureus (Table 1). In general, it was found that the growth inhibition zone diameter increased with increasing the concentration of essential garlic oil. Figure 1 shows the garlic essential oil inhibition zone diameter (corresponding to concentrations of 150 and 300 mg/mL) on Listeria innocua.

**Table 1**. The Disk Diffusion Agar (DDA) technique was utilized to discover the average inhibition zone diameters (mm) of Allium sativum essential oil against certain food-borne pathogens.

| | 37.5 mg/mL | 75 mg/mL | 150 mg/mL | 300 mg/mL |
|---|---|---|---|---|
| *Escherichia coli* | 16.10 ± 0.35 | 23.00 ± 0.40 | 24.10 ± 0.70 | 26.80 ± 0.85 |
| *Listeria innocua* | 17.6 ± 0.66 | 21.90 ± 0.83 | 24.30 ± 0.47 | 28.10 ± 0.40 |
| *Pseudomonas aeruginosa* | 19.50 ± 0.68 | 20.80 ± 0.75 | 24.20 ± 0.45 | 27.90 ± 0.50 |
| *Staphylococcus aureus* | 20.20 ± 0.58 | 26.00 ± 0.64 | 28.80 ± 0.95 | 31.70 ± 0.55 |

The results of the minimum inhibitory concentration of essential garlic oil on Listeria innocua, Staphylococcus aureus, Escherichia coli, and Pseudomonas aeruginosa are shown in Figure 2. The Allium sativum essential oil has a minimum bactericidal concentration (MBC) ≥ 512.

The results showed that gram-negative bacteria Pseudomonas aeruginosa and Escherichia coli were the most resistant bacteria to essential garlic oil. The most susceptible microbial strain to essential garlic oil was Staphylococcus aureus. It was found that the minimum bactericidal concentration of garlic essential oil in all studied bacteria was greater than the minimum inhibitory concentration. The results of gas chromatographic analysis of essential garlic oil are given in Figure 3.

Based on the results of gas chromatography, nine compounds were discovered in garlic essential oil (Figure 4). In general, these identified compounds constituted 94.54% of the total essential oil components.

Phenolic compounds of essential garlic oil were evaluated using Folin-Ciocalteu reagent. The absorption of essential garlic oil was compared with the uptake of gallic acid, and the findings revealed that the total phenol content of essential garlic oil was equal to 0.53 mg gallic acid per gram of essential oil. The amount of flavonoid compounds in essential garlic oil was measured by the aluminum trichloride colorimetric method. The findings revealed that the total flavonoid content of essential garlic oil was equal to 0.24 mg quercetin per gram of essential oil, and the antioxidant activity based on the percentage of free radical scavenger DPPH was equal to 80%. Today, due to the resistance of pathogenic microorganisms to drugs and antibiotics, many studies have been conducted to find suitable alternatives to these drugs. The number of studies conducted in this field is increasing day by day, and many researchers are trying to find compounds with minimal side effects and maximum ability to

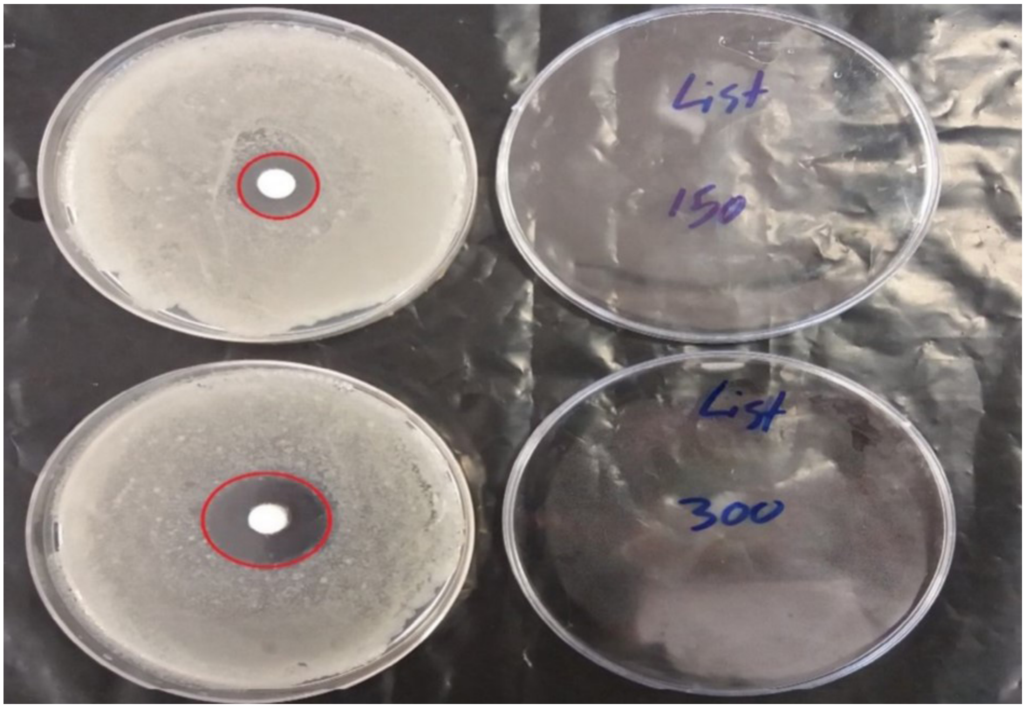


**Figure 1**. The diameter of the inhibitory zone on Listeria innocua (at 300 and 150 mg/mL concentrations) of Allium sativum essential oil (in mm).

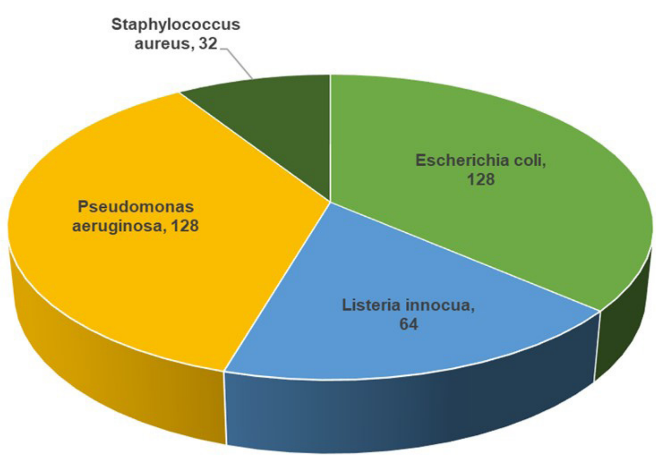


**Figure 2**. The Minimum Inhibitory Concentration (MIC) of Allium sativum essential oil on several foodborne pathogens.

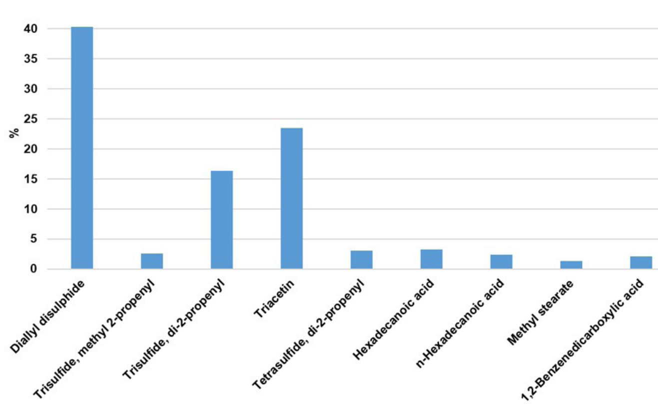


**Figure 3**. Chemical constituents of the essential oil of Allium sativum.

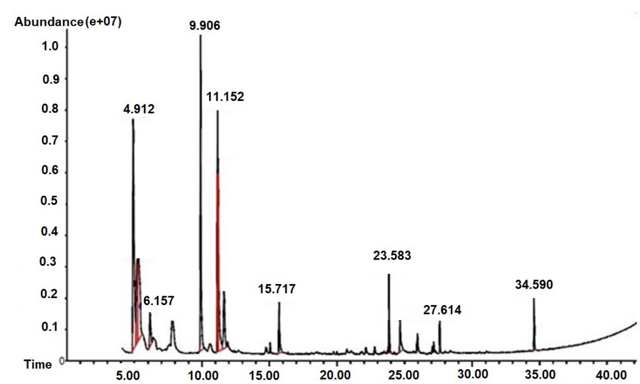


**Figure 4**. Chromatogram of the essential oil of Allium sativum.

fight against pathogenic microorganisms. The findings of this investigation revealed that the highest resistance to the essential oil was related to gram-negative Escherichia coli. Staphylococcus aureus showed the highest susceptibility to the essential oil. Gram-positive bacteria (Listeria innocua and Staphylococcus aureus) were generally more susceptible to gram-negative bacteria (Sodomonas aeruginosa, Escherichia coli).

## 4 Conclusion

The findings of this investigation revealed that essential oil has less antimicrobial effect than garlic extract, and in higher concentrations, an inhibitory and bactericidal effect was observed for essential garlic oil. According to the findings of our study, essential garlic oil had a larger zone diameter on Staphylococcus aureus and Escherichia coli bacteria. Many researchers have related the reason for this to differences in sex, species, place of plant growth, time of plant collection, soil type, climatic conditions, etc. In this research, increasing the concentration of essential garlic oil increased the growth inhibition zone diameter for all pathogenic microorganisms. Theoretically, the growth inhibition zone diameter is a reaction to the concentration of the active substance in the essential oil or antimicrobial agent. The growth inhibition zone diameter for Staphylococcus aureus (the most sensitive bacterium against garlic essential oil) increased from 20.20 mg to 31.70 mm at a concentration of 37.5 mg/mL to 300 mg/mL. According to our study, essential garlic oil had antimicrobial activity on all pathogenic microorganisms. However, the antibacterial activity of garlic essential oil on gram-positive bacteria was much higher than that of gram-negative bacteria. The results of chemical tests (total phenol, flavonoids, and antioxidant potential) of essential garlic oil showed that garlic has the potential source to be employed in food preservatives and medicinal compounds, although it is necessary to perform various tests in animal and human models. The mass spectrometer, diallyl disulfide compound with 40.3%, was the highest composition of essential garlic oil.

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