Original Article

The effect of Spearmint, Oregano, and Thyme Extracts on Biofilm Formation by *Listeria Monocytogenes, Escherichia Coli O157: H7,* and *Salmonella Typhimurium*

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ABSTRACT

The formation of bacterial biofilm on surfaces associated with food processing is of particular concern. Due to the health concerns associated with the production of biofilm on food-related surfaces and the increase in antimicrobial resistance in pathogenic bacteria, the present study aimed to investigate the anti-biofilm effects of oregano, spearmint, and thyme extracts against biofilms of Listeria monocytogenes, Escherichia coli O157: H7, and Salmonella typhimurium. Spearmint, oregano, and thyme plants were freshly prepared, dried, and ground. The aqueous and ethanolic extracts of the plants were extracted by soaking. The amount of phenolic compound of the aqueous and ethanolic extracts was evaluated by spectrophotometric method. The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were determined. The biofilm inhibition and destruction by the extracts were studied using the microdilution method. The results showed that the highest amount of phenolic compounds among ethanolic and aqueous extracts belonged to oregano and thyme extracts, respectively. The results also showed that the lowest effective concentration of the extracts on L. monocytogenes was by thyme aqueous extract with MIC and MBC of 1.8 and 2%, respectively, and for oregano ethanolic extract was 1.2 and 1.4%. The most significant biofilm inhibitory effect on L. monocytogenes, S. typhimurium, and E. coli O157: H7 was observed for thyme aqueous extract and oregano ethanolic extract. The results of the present study indicate that aqueous and ethanolic extracts of spearmint, oregano, and thyme plants have inhibitory and disruptive effects on biofilm formation by pathogenic bacteria. Therefore, these natural antimicrobial compounds can be used to control and prevent biofilm formation in the food industry.

Keywords: Biofilm, *Salmonella Typhimurium, Listeria Monocytogenes, Escherichia coli* O157: H7, Oregano, Thyme, Spearmint.

1. Introduction

Biofilm is ubiquitous and can be found in various environments including living tissues, natural aquatic systems, inanimate surfaces, food processing equipment, food contact surfaces, water system piping, and medical devices (1). The concept of biofilm was first proposed in 1971 by Marshall et al (2). Bacteria can switch between two distinct "lifestyles": a motile planktonic unicellular state and a biofilm state. A biofilm is a microbial community of cells embedded in an extracellular matrix that includes adhesives, exopolysaccharides, proteins, and DNA (3). Biofilms exist on most surfaces in the environment (1). The current definition of a bacterial biofilm is an enclosed community of cells that are self-producing in a matrix and adhere to abiotic or biotic surfaces. Biofilms form a protected state that allows survival under adverse environmental conditions. They may also have structures such as channels that allow nutrients to enter (4). It has been reported that eDNA and intracellular junctions of EPS act as a barrier to the penetration of several antimicrobial agents (2). The structural role of the extracellular matrix (ECM) contributes to the durability of biofilms in industry. Biofilms can form rapidly and spontaneously by bacteria on various surfaces, including food, metals, rubber, plastics, glass, cement and wood (1). By trapping nutrients and enzymes, biofilms can help create a genetic habitat for bacteria and make them resistant to antimicrobial agents. (5, 6). Salmonella typhimurium (S. typhimurium), Listeria monocytogenes (L. monocytogenes), and Escherichia coli O157: H7 (E. coli O157:H7) are significant foodborne pathogens that have the capacity to produce biofilms on surfaces associated with food. Numerous studies have demonstrated that Salmonella can form biofilms on non-living surfaces, including plastic, rubber, cement, glass, and stainless steel (1). L. monocytogenes has been observed to adhere and form biofilms on the surface of food processing equipment, including polystyrene, stainless steel, polymer, plastic, Teflon, and rubber (7). Furthermore, L. monocytogenes has been observed to thrive at low temperatures, and its capacity to form biofilms renders it challenging to remove during cleaning procedures (8). Numerous studies have indicated the existence of E. coli biofilms at all stages of food processing and production, contaminating food and causing foodborne illness (9). It is imperative for the food industry to prioritize the prevention of bacterial biofilms, encompassing both spoilage bacteria and pathogens, to ensure food safety. A failure to do so can lead to an increase in the resistance of biofilm bacteria to stress and disinfectants, exacerbating the already critical challenges posed by these microorganisms. Frequent contamination and rapid degradation of food by biofilm cells pose a significant food safety risk and threaten the health of consumers. Biofilms on surfaces and food processing equipment can readily contaminate final products, potentially resulting in foodborne infections or intoxications in consumers. The cells comprising the biofilm exhibit heightened resistance to heat, desiccation, acidic environments, salinity, antimicrobial agents, and food preservatives when compared with their planktonic counterparts. This attribute of bacterial biofilms underscores their status as a substantial threat to human and animal health (10, 8). One proposed method for controlling or eliminating biofilms involves regulating the output pump in bacterial cells. Bacteria employ diverse pump mechanisms to expel toxins and metabolic waste products. The activity of these pumps can lead to resistance to chemicals, including antibiotics, which can result in the emergence of strains resistant to multiple drugs (11). The eradication of biofilms poses a significant challenge for the food industry, as the microorganisms present within biofilms have developed a high degree of resistance to the conventional antimicrobial treatments currently employed in food production (12). Recent research suggests that at least 65% of bacteria causing infection and 70% of chronic infections in humans can be of biofilm origin (13). Plant extracts have been shown to possess antimicrobial properties and are recognized worldwide as potential sources of new antimicrobial compounds, particularly against bacterial pathogens. These extracts have been explored as a potential alternative in food preservation and the treatment of infectious diseases (14). Studies have demonstrated that phenolic compounds found in plants, such as carvacrol and thymol, exhibit high antioxidant and antimicrobial activity. The disruption of cytoplasmic integrity by phenolic compounds leads to the destruction of the bacterial outer membrane, which ultimately results in increased permeability of the membrane and the death of the bacteria (12, 3, 15). The present study was designed and implemented to investigate the effect of aqueous and ethanol extracts of spearmint, oregano, and thyme plants on the biofilm of Listeria monocytogenes, Escherichia coli O157: H7, and Salmonella typhimurium.

2. Materials and Methods

2.1. Preparation of Extracts

Fresh plants, including spearmint (Mentha spicata), oregano (Mentha pulegium), and thyme (Thymus vulgaris), were prepared, dried in the shade, and then ground. The extraction process involved the preparation of an aqueous extract by adding 500 milliliters of distilled water to 50 grams of plant powder in a 1-liter jar. To obtain an alcoholic extract, 500 milliliters of 96% ethanol were added to the same quantity of plant powder. The mixture was then subjected to a 24-hour agitation process in a shaker and subsequently maintained in a state of darkness for a duration of 48 hours at ambient temperature. Following this, the mixture underwent filtration and centrifugation thrice at 4000 rpm for 5 minutes, and was subsequently passed through Whatman filter paper. The aqueous and ethanolic extracts were then placed in an oven at a temperature of 40°C until they were dehydrated and dried. To reconstitute the extracts, 2 grams of the extract powder were dissolved in a beaker with 20 mL of sterile distilled water. The extract was subsequently filtered through a 0.45

µm head syringe filter under sterile conditions. The extracts were then stored in sterile and dark glass containers (3).

2.2. Determining the Phenolic Compounds of the Extracts

The spectrophotometric method was employed to ascertain the total phenolic compounds present in the extracts. This was achieved by utilizing a UV-VIS spectrophotometer in conjunction with the Folin-Ciocalteu reagent. To this end, 2 mL of 10% Folin-Ciocalteu reagent was added to 0.5 mL of extract. After a period of five minutes, 2 mL of 5% sodium carbonate solution was added. The spectrophotometer was then utilized to measure the absorbances of the samples after a two-hour period at a wavelength of 760 nanometers in comparison to a blank sample. Utilizing a calibration curve derived from gallic acid standards, the phenolic content was determined in gallic acid equivalents per gram of dry extract (16).

2.3. Determination of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) of the Extracts

S. typhimurium and L. monocytogenes, (bacterial bank of food hygiene laboratory of Shahrekord University) and E. coli O157:H7 (ATCC35218), obtained from microbiology laboratory bank of Faculty of Veterinary Medicine, University of Tehran, were used to determine the inhibitory concentration of the extracts. Microdilution method was used in TSB medium. Different percentages of extract were added to the wells. Each well was inoculated with 10^6/ml of bacteria. One well contained only 200 µL of culture medium (control), one well contained 100 µL of extract and 100 µL of TSB, and another well contained 190 µL of TSB and 10 µL of bacteria. The microplates were then incubated at 37°C for 24 hours. The first concentration in the well without turbidity was considered the minimum inhibitory concentration (MIC). To determine the MBC, 0.1 mL from the MIC well and the subsequent wells were cultured on the plate count agar medium and incubated at 37°C for 24 hours. The concentration of the first plate without bacterial growth was determined as the MBC (17). The tests were performed in three replicates.

2.4. Examining the Inhibition of Biofilm Production by the Extracts

The bacterial strain was first inoculated in the TSB medium and incubated at 37°C for 24 hours. Subsequently, the mixture was subjected to centrifugation at 4000 rpm for a duration of 5 minutes. Thereafter, the upper layer of the mixture was meticulously extracted using a sterile Pasteur pipette. Three milliliters of sterile phosphate buffer solution (PBS) was then added to the bacterial sediment and thoroughly mixed for one minute on a tube shaker to wash the bacterial cells. The mixture was subsequently subjected to a second centrifugation step for five minutes. Thereafter, the bacterial sediment was resuspended in a sterile PBS solution, and the McFarland scale was employed to determine the concentration of the bacterial solution. 100 µL of bacterial solution was added to the microplate wells, with concentrations equal to the MIC of the extracts and greater than the concentrations added to the wells containing bacteria. In a separate row of the microplate, 100 µL of the equivalent percentage of MIC and more of the extract and 100 µL of PBS solution were added as a negative control. In the subsequent row, 100 microliters of bacteria and 100 microliters of PBS solution were added as a positive control of biofilm. In the subsequent row, 100 µL of sodium hypochlorite and 100 µL of PBS solution were added as a positive control. These tests were performed for all aqueous and alcoholic extracts. The microplate was then subjected to an incubation period at 37°C for a duration of 48 hours. Following the completion of the incubation period, the liquid in the microplate was meticulously drained using a sampler, and 200 µL of 1% crystal violet solution was added to all the wells. Following a 30-minute incubation at room temperature, the dye was completely drained, and the wells were washed twice with a solution of phosphate-buffered saline (PBS). Subsequently, 150 µL of 96% ethanol was added to the wells, and after 15 minutes, the contents of each well were meticulously transferred to a new microplate. The absorbances of the wells were subsequently measured using an ELISA reader at a wavelength of 620 nm. To calculate the inhibition of biofilm formation by the extracts, formula 1 was employed (18).

Formula 1: Percentage inhibition = 100 - [{OD600 nm experimental well with Ex / OD600 nm control well without Ex} × 100].

M: Percentage of biofilm formation destruction.

A: Mean optical absorbance of the sterile distilled water control.

B: Mean optical absorption of the culture medium control.

C: Mean optical absorption of the test well.

D: Mean optical absorbance of the extract control.

2.5. Determining the Biofilm Destruction by the Extracts

The bacterial samples were cultivated in Tryptic Soy Broth (TSB) medium for a duration of 24 hours at a temperature of 37°C. Subsequently, 1 mL of the bacterial suspension was mixed with 5 mL of sterile TSB medium, and 100 µL of this mixture was added to each well of the microplate. The microplate was then subjected to a 48-hour incubation period at 37°C. Subsequently, the medium's upper layer was carefully extracted, and the non-adherent bacterial cells were removed through a process of washing with a sterile PBS solution. To ascertain the effect of the extract on the biofilm, 100 µL concentrations equivalent to the MIC of the extracts and above were added to six rows of microplate wells. The seventh row was filled with sterile distilled water, and the eighth row was filled with 100 µL of sterile TSB. The microplate was then subjected to an incubation period at a temperature of 37°C for a duration of 24 hours. Subsequent to this, the contents of the wells were meticulously removed, and 200 µL of 1% crystal violet was added to all wells. Following a 30-minute incubation period, the crystal violet was meticulously removed from the wells. The wells were then washed twice with a solution

of phosphate-buffered saline (PBS). Subsequently, 150 µL of 96% ethanol was added to the wells. After a duration of 15 minutes, the absorbances of the wells were measured at a wavelength of 620 nm using an ELISA reader. Finally, the percentage of biofilm destruction in the presence of concentrations of extracts was calculated using the formula 2 (19).

Formula 2: M=100×{(A-B)-(C-D) / (A-B)}

M: Percentage of biofilm destruction.

A: Mean optical absorbance of the sterile distilled water control.

B: Mean optical absorbance of the culture medium control.

C: Mean optical absorbance of the test well.

D: Mean optical absorbance of the extract control.

2.6. Data Analysis

The data obtained from the tests were then subjected to analysis using Sigma Plot 12 statistical software, employing McNemar's test at a significant level of P<0.05.

3. Results

The results of the study demonstrated that the ethanolic extracts of the tested plants exhibited a higher phenolic content compared to the aqueous extracts. The findings further demonstrated that the highest recorded concentration of phenolic compounds was 268.2761 milligrams per liter (mg/L) in the ethanolic extract of oregano, while the lowest was 80.2581 mg/L in the aqueous extract of oregano (Table 1).

3.1. MIC and MBC of the Extracts for the Tested Bacteria

The minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of the extracts were evaluated against Listeria monocytogenes, Salmonella typhimurium, and Escherichia coli O157:H7. The ethanol extract of oregano demonstrated the most effective concentration against L. monocytogenes, with an MIC of 1.2% and an MBC of 1.4%. Moreover, the results demonstrated that among the aqueous extracts, thyme aqueous extract exhibited the lowest effective concentration against L. monocytogenes bacteria with an MIC of 1.8% and an MBC of 2%. For S. typhimurium, the MIC and MBC values for the thyme aqueous extract were 3% and 3.2%, respectively, while for oregano ethanol extract, they were 1.6% and 1.8%, respectively. In the case of E. coli O157:H7, the thyme aqueous extract exhibited the lowest effective concentration, with a MIC of 2.9% and an MBC of 3.1%, while the oregano ethanol extract demonstrated a MIC and MBC of 1.8% and 2%, respectively. The findings indicated that among the extracts, the thyme aqueous extract and oregano ethanol extract demonstrated the most significant effect at lower concentrations. As demonstrated in Table 2, a statistically significant discrepancy was observed between the MIC and MBC of diverse extracts for the examined bacteria (P<0.05). In summary, the findings indicate that the ethanolic extracts of the studied plants exhibited an inhibitory and lethal effect on the tested bacteria at lower concentrations compared to the aqueous extracts (Table 2).

3.2. Biofilm Inhibition

As indicated by the findings presented in Table 3, among the aqueous extracts, thyme extract exhibited the most significant inhibitory effect on biofilm formation, with a recorded 78% inhibition, while the ethanolic extract of oregano demonstrated the most pronounced inhibitory effect against L. monocytogenes among the ethanolic extracts, with a recorded 95% inhibition. The aqueous extract of thyme demonstrated a 74% prevention of S. typhimurium biofilm formation, while the ethanolic extract of oregano exhibited a 93% inhibition. Among the ethanolic extracts, the ethanolic extract of oregano exhibited the most significant biofilm inhibition effect on E. coli O157:H7, with 91% inhibition. Similarly, among the aqueous extracts, the aqueous extract of thyme demonstrated the highest biofilm inhibition, with 72% inhibition. In comparison with the extracts, sodium hypochlorite exhibited the most pronounced inhibition of biofilm formation. The statistical test indicated that there was no significant difference between the biofilm inhibition effect of ethanol extracts and sodium hypochlorite, but the difference was significant for aqueous extracts and sodium hypochlorite (P < 0.05) (Table 3).

3.3. Destruction of Biofilm

The findings indicated that thyme aqueous extract (70%) and oregano extract (92%) exhibited the most significant impact on L. monocytogenes biofilm. Furthermore, the study revealed that ethanolic extracts exhibited a more pronounced ability to dismantle the biofilm of the tested bacteria in comparison to aqueous extracts. However, a statistically insignificant difference was observed between the aqueous and ethanolic extracts of spearmint and thyme with respect to their biofilm-destructive capabilities. However, a statistically significant difference was observed between the aqueous and ethanolic extracts of oregano compared to those of spearmint and thyme (P<0.05) (Table 4). The findings indicate that the eradication of biofilms formed by the tested bacteria is accomplished by aqueous and ethanolic extracts of thyme, spearmint, and oregano plants at concentrations exceeding the MBC. In general, ethanolic extracts of the studied plants exhibited a greater effect on the destruction of bacterial biofilms compared to aqueous extracts, particularly at lower concentrations. The aqueous extract of thyme and the ethanolic extract of oregano exhibited the most pronounced effect on complete biofilm destruction (Table 5). A statistically significant difference was observed between the aqueous extracts of the three plants in terms of their ability to destroy bacterial biofilms. Additionally, a statistically significant difference was identified between the ethanolic extract of spearmint and two other ethanol extracts (P<0.05).

Table 1. Phenolic compounds in aqueous and ethanolic extracts of Thyme, Spearmint, and Oregano.

		ABS (Absorbance)	**Phenolic concentration mg/L**
Extract	Blank	0	0
Aqueous	Oregano	0.71877	80.2581
	Thyme	1.2277	137.7259
	Spearmint	1.1344	127.1920
Ethanolic	Oregano	2.3840	268.2761
	Thyme	2.2575	253.9938
	Spearmint	1.7513	196.1421

Table 2. The MIC and MBC of the extracts for the tested bacteria (%).

Plant	**Aqueous extract**						**Ethanol extract**					
	L. monocytogenes		***S. typhimurium***		***E. coli* O157:H7**		***L. monocytogenes***		***S. typhimurium***		***E. coli* O157:H7**	
	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC	MIC	MBC
Spearmint	2.8^{a}	3.1^{a}	4.5^{a}	4.8^{a}	4.5^{a}	4.8^{a}	2.2^{a}	2.5^{a}	2.8^{a}	3.1^{a}	3^{a}	3.3^{a}
Oregano	4.5^{b}	5^{b}	6^{b}	6.5^{b}	6^{b}	6.5^{b}	1.2^{b}	1.4^{b}	1.6^{b}	1.8^{b}	1.8^{b}	2^{b}
Thyme	1.8^{c}	2^{c}	3^{c}	3.2^{b}	2.9^{c}	3.1^{c}	1.5^{b}	1.7^{b}	1.8^{b}	2^{b}	2^{b}	2.2^{b}

Different letters in each column indicate the statistically significant differences (P<0.05).

Table 3. Percentage of biofilm formation inhibition by extracts for *L. monocytogenes*, *S. typhimurium,* and *E.coli* O157:H7.

Plant	**Aqueous extract**			**Ethanol extract**		
	L. monocytogenes	***S. typhimurium***	***E. coli* O157:H7**	***L. monocytogenes***	***S. typhimurium***	***E. coli* O157:H7**
Spearmint	73^{a}	70^{a}	67^{a}	84^{a}	81^{a}	78^{a}
Thyme	78^{a}	74^{a}	72^{a}	89^{a}	85^{a}	81^{a}
Oregano	60^{b}	54^{b}	52^{b}	95^{b}	93^{b}	91^{b}
Sodium hypochlorite	88^{c}	92^{c}	80^{c}	88^{bc}	92^{c}	80^{a}

Different letters in each column indicate the statistically significant differences (P<0.05).

Table 4. Biofilm destruction by aqueous extract and ethanolic extract on *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7 (%).

Plant	**Aqueous extract**			**Ethanolic extract**		
	L. monocytogenes	***S. typhimurium***	***E. coli* O157:H7**	***L. monocytogenes***	***S. typhimurium***	***E. coli* O157:H7**
Spearmint	65^{a}	60^{a}	58^{a}	73^{a}	67^{a}	65^{a}
Thyme	70^{a}	65^{a}	61^{a}	85^{b}	82^{b}	79^{b}
Oregano	50^{b}	48^{b}	45^{b}	92^{c}	90^{c}	88^{c}

Different letters in each column indicate the statistically significant differences (P<0.05).

Table 5. The concentration of aqueous and ethanolic extracts of thyme, oregano, and spearmint in destruction of *S. typhimurium*, *L. monocytogenes*, and *E. coli* O157:H7 biofilms (%).

Plant	**Aqueous extract**			**Ethanol extract**		
	L. monocytogenes	***S. typhimurium***	***E. coli* O157:H7**	***L. monocytogenes***	***S. typhimurium***	***E. coli* O157:H7**
Spearmint	3.3^{a}	5^{a}	5^{a}	2.7^{a}	3.3^{a}	3.4^{a}
Oregano	5.2^{b}	6.8^{b}	6.8^{b}	1.6^{b}	3^{b}	2.1^{b}
Thyme	2.2^{c}	3.2^{c}	3.3^{c}	1.9^{b}	2.2^{b}	2.3^{b}

Different letters in each column indicate the statistically significant differences (P<0.05).

4. Discussion

Biofilms are of particular concern in the context of food safety due to their tendency to accumulate on foodstuffs and surfaces. The presence of biofilms can reduce the shelf life of food products and even facilitate the transmission of infectious diseases to humans. Consequently, the identification of methodologies to impede the formation and dissolution of biofilms has emerged as a pivotal subject in contemporary research endeavors. Phenolic compounds derived from plant sources have been shown to exert a detrimental effect on various pathogenic bacterial strains. The present study investigates the inhibitory and bactericidal effects of aqueous and ethanolic extracts of spearmint, oregano, and thyme plants on the biofilm of L. monocytogenes, E. coli O157:H7, and S. typhimurium. The findings of this study indicate that ethanolic extracts possess a higher phenolic content compared to aqueous extracts, a phenomenon that is likely attributable to the lower solubility of these compounds in water. These outcomes align with the findings of Mazarai et al. (20), who reported that among four solvents (water, methanol, acetone, and ethanol), methanol exhibited the highest phenol content, while water exhibited the lowest. The extraction of these compounds is contingent on several factors, the most significant of which are the solvent and extraction method. The selection of an appropriate solvent and extraction method is contingent on the particular characteristics of the plant material and its constituent components. As demonstrated by Hanachi et al. (21), an ethanol/methanol 70% solvent ratio of 1:1 was identified as the optimal extraction medium for phenolic compounds. The MIC and MBC of the extracts for Escherichia coli O157:H7, Listeria monocytogenes, and Salmonella typhimurium were obtained with the lowest percentage of aqueous thyme extract and ethanolic extract of oregano. Dauqan et al. (5) investigated the effect of aqueous thyme extract on E. coli O157:H7 and reported MIC values of 2.9% and MBC of 3.1%. Additionally, Damelian et al. evaluated the impact of spearmint essential oils on the growth and survival of various foodborne pathogen bacteria, including B. cereus, S. typhimurium, L. monocytogenes, and Y. enterocolitica. Their findings indicated that the essential oils, despite being in low concentrations, exhibited antibacterial properties, thereby impeding bacterial growth. In a separate study, Broumand et al. (23) found that a film containing Shirazi thyme essential oil at a concentration of 250 ppm could inhibit the growth of S. typhimurium, S. aureus, and E. coli O157:H7. The findings of the present study further demonstrated that the aqueous and ethanolic extracts of the examined plants exhibited a more pronounced effect on L. monocytogenes compared to the other two gram-negative bacteria. In a separate study, Fatemeh Akhwan et al. (24) demonstrated that thyme extracts had the most antimicrobial effect on gram-positive bacteria like Bacillus cereus, L. monocytogenes, and Staphylococcus aureus. In the context of the present study's findings, ethanolic extracts exhibited a more pronounced capacity to impede biofilm formation in comparison to aqueous extracts. In comparison to sodium hypochlorite, aqueous extracts exhibited a reduced inhibitory effect on bacterial biofilm formation, while ethanolic extracts demonstrated comparable or enhanced performance in suppressing biofilm production. However, previous studies have demonstrated that despite the enhanced efficacy of disinfectants on biofilm formation, bacteria within the biofilm rapidly develop resistance to these compounds (25). A multitude of studies have indicated that bioactive compounds, such as carvacrol and thymol, in low concentrations, can significantly inhibit biofilm formation by bacteria (26). In a related finding, Hyung Lee et al. (27) reported that 16 Asian medicinal plants exhibited high anti-biofilm activity against EHEC without affecting planktonic cell growth. Zoya Samoilova (3) found that yarrow's alcoholic extract significantly reduced biofilm formation by E. coli. Cabarkapa et al. (28) found that carvacrol and thymol inhibited the biofilm formation of S. Enteritidis at the lowest concentration. The collective findings of these studies suggest that plant compounds exert their biofilm control effect through the regulation of genes and proteins involved in matrix mobility and exopolysaccharide (EPS) production (29). The efficacy of plant compounds in controlling biofilms has been attributed to their impact on genes involved in the production of matrix proteins and exopolysaccharides (EPS) (26, 6). A separate study has demonstrated that phenol compounds, such as carvacrol, impede the expression of genes associated with bacterial adhesion to surfaces, including aggR, pic, aap, aggA, and eae (6). Sumrani et al. (8) demonstrated that the MIC values of onion extract inhibited the primary cell adhesion of bacteria by 77%, while cinnamon and garlic extract completely inhibited adhesion. In a separate study, Davila-Aviña et al. (29) reported that gallic acid, a plant compound, hinders E. coli biofilm formation, while tannic acid and methylgallate promote it. The findings of the present study demonstrate that the most efficacious destruction of bacterial biofilms is attained by ethanolic extracts. Specifically, the ethanolic extract of oregano exhibited the most pronounced destructive effect on the biofilms formed by the tested bacteria. Among the aqueous extracts, thyme extract exhibited a significant capacity to destroy bacterial biofilms. A comparison of the effects of the tested extracts reveals that they are more effective in inhibiting biofilm production than in eradicating it. This observation aligns with previous studies highlighting the heightened resistance of preformed biofilms to antimicrobial agents and plant extracts (8). Guo et al. (30) observed a decrease in the thickness and density of S. aureus biofilms upon exposure to phenolic compounds, as evidenced by scanning electron microscopy. These compounds have been shown to possess a bactericidal effect on biofilm bacteria, resulting in the removal of polysaccharides and proteins from mature biofilms and subsequent biofilm destruction. In light of the pivotal role biofilms play in the food industry, where they facilitate the

adhesion of pathogenic and spoilage bacteria to surfaces in contact with food, the removal of biofilms poses a significant challenge. The present study thus explores the potential of plant extracts from spearmint, oregano, and thyme to combat bacterial biofilms in the food industry.

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Authors' Contribution
Concept and design of the study: M.B
Collection of data: L.A.A
Analysis and interpretation of data: M.B and H.M
Drafting of the manuscript: L.A.A
Critical revision of the manuscript for important
Intellectual content: M.B
Statistical analysis: H.M
Administrative, technical, and material support: L.A.A

Ethics
It is evident that no particulars of this scholarly composition pertain to the use of experimental animals or the specific human diseases that necessitate the publication and approval of publishing ethics.

Conflict of Interest
The authors declare that they have no conflict of interest.

Data Availability
All data generated or analyzed during this study are included in this published article.

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