The effect of spearmint, oregano, and thyme extracts on biofilm formation by *Listeria monocytogenes, Escherichia coli O157: H7,* and *Salmonella typhimurium.*

Abstract

The formation of bacterial biofilm on surfaces related to food processing is of particular importance. Due to the health concerns associated with the production of biofilm on food-related surfaces and the increase of antimicrobial resistance in pathogenic bacteria, the present study aimed to investigate the anti-biofilm effects of oregano, spearmint, and thyme extracts against the biofilms of *Listeria monocytogenes*, *Escherichia coli* O157: H7, and *Salmonella typhimurium*.

Spearmint, Oregano, and Thyme plants were freshly prepared, dried, and ground. The hydro and ethanolic extracts of the plants were extracted by soaking. The amount of phenolic compound of hydro and ethanolic extracts was evaluated using the spectrophotometric method. The extracts' minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) were determined. The biofilm inhibition and destruction by the extracts were examined using the microdilution method.

The results showed that the highest amount of phenolic compounds among ethanolic and aqueous extracts belongs to oregano and thyme extracts, respectively. Also, the results 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-inhibiting effect on *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157: H7 was observed by the thyme aqueous extract and oregano ethanolic extract. Moreover, the highest amount of biofilm destruction was achieved by the 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 destructive effects on biofilm formation by pathogenic bacteria. Therefore, these natural antimicrobial compounds can be used to control and prevent biofilm formation in food industries.

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, non-living surfaces, food processing equipment, food contact surfaces, water system piping, and medical equipment (1). The concept of biofilm was first proposed by Marshall et al. in 1971 (2). Bacteria can switch between two distinct "lifestyles": a motile planktonic unicellular state and a biofilm state. A biofilm is a microbial community with cells embedded in an extracellular matrix, which includes adhesives, exopolysaccharides, proteins, and DNA (3).

Biofilms exist on most surfaces in the open air (1). The current definition of a bacterial biofilm is an enclosed community of cells that self-produce in a matrix and adhere to abiotic or biotic surfaces. Biofilms form a protected state that allows survival in unfavorable environmental conditions. They can also feature structures such as channels, which allow nutrients to enter (4). It has been reported that eDNA and intracellular junctions of EPS act as a barrier to the penetration of various antimicrobials (2). The structural role of ECM (Extra Cellular Matrix) helps to strengthen the durability of biofilms in industries.

Biofilms can form quickly and spontaneously by bacteria on various surfaces, including food, metals, rubber, plastic, glass, cement, and wood (1). By trapping nutrients and enzymes, biofilm can help create the genetic habitat of bacteria and make them resistant to antimicrobial agents. (5, 6).

Bacteria such as *Salmonella typhimurium* (*S. typhimurium)*, *Listeria monocytogenes* (L. *monocytogenes*), and *Escherichia coli* O157: H7 (*E. coli* O157:H7), are important foodborne pathogens that can produce biofilms on food-related surfaces. Various studies have shown that Salmonella can form biofilms on non-living surfaces such as plastic, rubber, cement, glass, and stainless steel (1). *L. monocytogenes* can adhere and form biofilms on the surface of food processing equipment, including polystyrene, stainless steel, polymer, plastic, Teflon, and rubber (7). Also, *L. monocytogenes* grows at low temperatures, and its ability to form biofilms is difficult to remove during the cleaning process (8). Numerous studies indicate that *E. coli* biofilms exist in all stages of food processing and production, contaminating food and causing foodborne illness (9). Preventing the formation of bacterial biofilm, including spoilage bacteria and pathogens, is a vital task in the food industry, otherwise, it increases the resistance of biofilm bacteria to stress, and disinfectants.

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 easily contaminate final products, resulting in food infection or intoxication in consumers. The cells in the biofilm are more resistant to heat, desiccation, acidic environment, salinity, antimicrobial agents, and food preservatives than their planktonic counterparts, therefore bacterial biofilms are a significant threat to human and animal health (10, 8). One of the ways to control or eliminate biofilm is to control the output pump in bacterial cells. Bacteria use different pump systems to drive toxins and waste metabolites out. The activity of the pumps can cause resistance to chemicals such as antibiotics, followed by the emergence of strains resistant to several drugs. (11). Biofilm eradication is a challenge for the food industry because the microorganisms

present in the biofilm have become very resistant to the conventional antimicrobial treatments currently used in the food industry (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 antimicrobial properties and are recognized worldwide as potential sources of new antimicrobial compounds, particularly against bacterial pathogens. It is used as a possible 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. Phenolic compounds disrupt cytoplasmic integrity, leading to the destruction of the outer membrane of bacteria, which ultimately results in increased permeability of the membrane and leads to 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. To prepare the extract, 50 grams of plant powder was poured into a 1-liter jar, then 500 ml of distilled water was added to prepare the aqueous extract, and 500 ml of 96% ethanol was added to prepare the alcoholic extract. The mixture was then placed in a shaker for 24 hours and kept in the dark for 48 hours at room temperature. After that, the mixture was filtered and centrifuged three times at 4000 rpm for 5 minutes, and passed through Whatman filter paper. The aqueous and ethanolic extracts were 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 then filtered through a 0.45 µm head syringe filter under sterile conditions. The extracts were kept in sterile and dark glass containers (3).

2.2. Determining the phenolic compounds of the extracts

The spectrophotometric method was used to determine the total phenolic compounds of the extracts by UV-VIS spectrophotometer using the Folin-Ciocalteu reagent. For this purpose, 2 mL of 10% Folin-Ciocalteu reagent was added to 0.5 mL of extract and after 5 minutes, 2 mL of 5% sodium carbonate solution was added. The absorbance of the samples was read after 2 hours at a wavelength of 760 nm against the blank. Using the gallic acid standard, a calibration curve was drawn, and the phenolic content was calculated 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 the food hygiene laboratory at Shahrekord University), as well as the *E. coli* O157:H7 (ATCC35218) obtained from the microbiology laboratory bank of the Faculty of Veterinary Medicine, University of Tehran, were used to determine the inhibitory concentration of the extracts. The microdilution method was employed in the TSB medium. Different percentages of the extract were added to the wells. Each well was inoculated with 10^6/ml of the 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 the TSB and 10 µL of bacteria. The microplates were then incubated at 37°C for 24 hours. The first concentration of the well without turbidity was considered as the minimum inhibitory concentration (MIC). To determine MBC, 0.1 ml from the MIC well and after 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 MBC (17). The tests were performed in three repetitions.

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. The mixture was then centrifuged at 4000 rpm for 5 minutes, and the supernatant was carefully drained using a sterile Pasteur pipette. Three milliliters of sterile phosphate buffer solution (PBS) was added to the bacterial sediment and thoroughly mixed for 1 minute on a tube shaker to wash the bacterial cells. The mixture was centrifuged again for 5 minutes, and after draining the supernatant, the concentration of McFarland was created by adding PBS solution. 100 µL of bacterial solution was added to the microplate wells and concentrations equal to the MIC of the extracts and more than was added to the wells containing bacteria. In another 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 next row, 100 microliters of bacteria and 100 µL of PBS solution were added as a positive control of biofilm. In the next row, 100 µL of sodium hypochlorite and 100 µL of PBS solution were added as a positive control. The tests were performed for all aqueous and alcoholic extracts. The microplate was then incubated at 37°C for 48 hours. After incubation time, the liquid in the microplate was carefully drained using a sampler, and 200 µL of 1% crystal violet solution was added to all the wells. After 30 minutes at room temperature, the dye was completely drained and the wells were washed twice with PBS solution. Then, 150 µL of 96% ethanol alcohol was added to the wells, and after 15 minutes, the contents of each well were carefully transferred to a new microplate. The absorbance of the wells was read using an ELISA reader at a wavelength of 620 nm, and formula 1 was used to calculate the inhibition of biofilm formation by the extracts (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 bacteria were incubated in the TSB medium for 24 hours at 37°C. After that, 1 mL of medium containing bacteria was mixed with 5 mL of sterile TSB medium, and then 100 µL was added to each microplate well. The microplate was incubated at 37°C for 48 hours. The supernatant was then slowly removed, and the non-adherent cells were removed by washing with the sterile PBS solution. To determine the effect of the extract on the biofilm, 100 µL concentrations equivalent to the MIC of the extracts and more were added to six rows of microplate wells. Sterile distilled water was added to the seventh row and 100 µl of sterile TSB was added to the eighth row. Then, the microplate was incubated at 37°C for 24 hours. After that, the contents of the wells were slowly removed, and 200 µL of 1% crystal violet was added to all wells. After 30 minutes, crystal violet was slowly removed from the wells. The wells were then washed twice with PBS solution. Subsequently, 150 µL of 96% ethanol alcohol was added to the wells. After 15 minutes, the absorbance of the wells was read 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: $M=100\times\{(A-B)-(C-D) / (A-B)\}$

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 analyzed by Sigma Plot 12 statistical software using McNemar's test at a significant level of $P < 0.05$.

3. Results

Over all the results revealed that the ethanolic extracts of the tested plants contain more phenolic content than the aqueous extracts. The results also showed that the highest concentration of phenolic compounds was 268.2761 mg/L in the ethanol extract of oregano, while the lowest was 80.2581 mg/L in the aqueous extract of oregano. (**Table 1**)

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

3.1. MIC and MBC of the extracts for the tested bacteria

The MIC and MBC of the extracts were evaluated against *L. monocytogenes*, *S. typhimurium*, and *E. coli* O157:H7. The ethanol extract of oregano exhibited the lowest effective concentration against *L. monocytogenes* with a MIC of 1.2% and MBC of 1.4%. Also, the results showed that among aqueous extracts, thyme aqueous extract had the lowest effective concentration on *L. monocytogenes* bacteria with MIC 1.8% and MBC 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. For *E. coli* O157:H7, the thyme aqueous extract had the lowest effective concentration, with a MIC of 2.9% and MBC of 3.1%, and for oregano ethanol extract, the MIC and MBC were 1.8% and 2%, respectively. The results showed that among the extracts, the thyme aqueous extract and oregano ethanol extract exhibited the greatest effect in lower concentrations. As shown in Table 2, there is a statistically significant difference between MIC and MBC of different extracts for the tested bacteria ($P < 0.05$). In general, the results showed that the ethanolic extracts of the studied plants had an inhibitory and lethal effect on the tested bacteria in a lower concentration than the aqueous extracts. (**Table 2**)

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 ($P<0.05$)

3.2. Biofilm inhibition

As the results present in Table 3, among the aqueous extracts, the thyme extract had the highest effect on inhibiting biofilm formation with 78%, and the ethanolic extract of oregano with 95% inhibition among the ethanolic extracts on *L. monocytogenes*. Aqueous extract of thyme prevented the formation of biofilm by *S. typhimurium* by 74% and ethanolic extract of oregano by 93%. Among the ethanolic extracts, the ethanolic extract of oregano with 91%, and among the aqueous extracts, the aqueous extract of thyme with 72%, showed the highest biofilm inhibition effect on *E. coli* O157:H7. In comparison with the extracts, the highest inhibition of biofilm formation was observed by sodium hypochlorite. The statistical test showed that there is no significant difference between the effect of biofilm inhibition by ethanol extracts and sodium hypochlorite, but the difference was significant for aqueous extracts and sodium hypochlorite ($P<0.05$), (**Table 3**).

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 ($P<0.05$)

3.3. Destruction of biofilm

The results showed that thyme aqueous extract (70%) and oregano extract (92%) have the highest effect on *L. monocytogenes* biofilm. The results also show that ethanolic extracts destroy the biofilm of tested bacteria to a greater extent than aqueous extracts. There was no statistically significant difference between the aqueous and ethanolic extracts of spearmint and thyme in terms of biofilm destruction. However, there was a statistically significant difference between the aqueous and ethanolic extracts of oregano compared to those of spearmint and thyme ($P < 0.05$). (**Table 4**)

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 ($P<0.05$)

The results show that the destruction of biofilms formed by tested bacteria is achieved by aqueous and ethanolic extracts of thyme, spearmint, and oregano plants at concentrations higher than the MBC. In general, ethanolic extracts of the studied plants at lower concentrations compared to aqueous extracts destroyed the bacterial biofilms. The aqueous extract of thyme and the ethanolic extract of oregano had the greatest effect on complete biofilm destruction (**Table 5**). There is a significant difference between the aqueous extracts of all three plants in the destruction of bacterial biofilms; also, there is a statistically significant difference between the ethanolic extract of spearmint and two other ethanol extracts ($P < 0.05$).

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 crucial in terms of food safety due to their accumulation in food and surfaces. The presence of biofilms can reduce the shelf life of food products and even transmit infectious diseases to humans. Identifying ways to prevent the formation and destruction of biofilms is a significant topic in recent research. Phenolic compounds of plants have destructive effects on pathogenic bacteria. In the present study, the effect of aqueous and ethanolic extracts of spearmint, oregano, and thyme plants on inhibiting and destroying the biofilm of *L. monocytogenes*, *E. coli* O157:H7, and *S. typhimurium* was investigated. According to the results, ethanolic extracts have a higher phenolic content compared to aqueous extracts, likely due to the lower solubility of these compounds in water. These results are consistent with the research of Mazarai et al. (20), based on their findings, four solvents (water, methanol, acetone, and ethanol), it was found that methanol had the highest amount of phenol and water had the lowest amount of phenolic compounds. The extraction of these compounds depends on several factors, with the most important being the solvent, and extraction method. The choice of solvent and extraction method depends on various parts of a plant as well as its ingredients. Hanachi et al. (21) showed that an ethanol/methanol 70% solvent with a 1:1 ratio is the most suitable solvent for extracting phenolic compounds.

The MIC and the 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%. Also, Damelian et al. evaluated the effect of spearmint essential oils on the growth and survival of some foodborne pathogen bacteria, including *B. cereus*, *S. typhimurium*, *L. monocytogenes*, and *Y. enterocolitica*. They found that the low percentage of spearmint essential oils inhibited the growth of bacteria (22). Broumand et al. (23) revealed that a film containing Shirazi thyme essential oil with a concentration of 250 ppm inhibited the growth of *S. typhimurium*, *S. aureus*, and *E. coli* O157:H7. The results of the present study also showed that the aqueous and ethanolic extracts of the examined plants had a greater effect on *L. monocytogenes* compared to the other two gram-negative bacteria. 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*.

Regarding the results of the present study, ethanolic extracts showed a greater ability to inhibit biofilm formation compared to aqueous extracts. Compared to sodium hypochlorite, aqueous extracts showed less inhibitory effect on bacterial biofilm formation, while ethanolic extracts showed similar or better performance in inhibiting biofilm production. Previous studies have shown that despite the better effect of disinfectants on biofilm formation, the bacteria in the biofilm quickly become resistant to these compounds (25).

Several studies have indicated that bioactive compounds, such as carvacrol and thymol, in low concentrations significant effect in inhibiting biofilm formation by bacteria (26). Hyung Lee et al. (27) reported that 16 Asian medicinal plants showed high anti-biofilm activity against EHEC without inhibiting 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 findings suggest that plant compounds exert biofilm control through the regulation of genes and proteins involved in matrix mobility and exopolysaccharide (EPS) production (29). The other studies demonstrate that the high ability of plant compounds to control biofilms is due to their effect on genes encoding the production of matrix proteins and exopolysaccharides (EPS) (26, 6). The other study has shown that phenol compounds, such as carvacrol, prevent the expression of genes related to bacterial adhesion to surfaces, including *aggR*, *pic*, *aap*, *aggA*, and *eae* (6). Sumrani et al. (8) showed that the MIC values of onion extract inhibited the primary cell adhesion of bacteria by 77%, while cinnamon and garlic extract completely inhibited adhesion. Davila-Aviña et al. (29) reported that among plant compounds, gallic acid inhibits *E. coli* biofilm formation, whereas tannic acid and methylgallate encourage biofilm production.

Also, the results of the present study show that the most effective destruction of bacterial biofilms is achieved by ethanolic extracts. Ethanolic extract of oregano had the most destructive effect on the biofilms formed by the tested bacteria. Among the aqueous extracts, thyme extract significantly destroyed bacterial biofilms. A comparison of the effects of the tested extracts shows that they inhibit biofilm production to a greater extent than they destroy it. Previous studies have also shown that preformed biofilms show more resistance to antimicrobial agents and plant extracts (8). Guo et al. (30) using scanning electron microscopy found that the thickness and density of *S. aureus* biofilms decreased when exposed to phenolic compounds. These compounds have a bactericidal effect on biofilm bacteria, removing polysaccharides and proteins from mature biofilms and causing biofilm destruction.

Considering the importance of biofilms in the food industry due to the attachment of pathogenic and spoilage bacteria to surfaces in contact with food, removing biofilms is considered an important challenge. Therefore, according to the valuable properties of spearmint, oregano, and thyme plants shown in this research, the extracts of these plants can be used to inhibit and destroy 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

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Statistical analysis: H.M

Administrative, technical, and material support: L.A.A

Ethics

None aspects of this paper related to experimental animals or specific human diseases that require the publication and approval of publishing ethics.

Conflict of interest

The authors declare that they have no conflict of interest.

Data availability statement

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

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