Journal of Food Science and Technology (Iran)

Homepage:www.fsct.modares.ir

Scientific Research

Investigating the antimicrobial and antibiofilm potential of Melittin peptide against *Escherichia coli* and *Staphylococcus aureus*

Arezou Rouhi [1], Fereshteh Falah [1], Marjan Azghandi [2], Behrooz Alizadeh Behbahani [3], Farideh Tabatabaei-Yazdi [1*], Salam A. Ibrahim [4], Alireza Vasiee [5]

1- Department of Food Science and Technology, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

2- Department of Animal Science, Faculty of Agriculture, Ferdowsi University of Mashhad, Mashhad, Iran

3 -Department of Food Science and Technology, Faculty of Animal Science and Food Technology, Agricultural Sciences and Natural Resources University of Khuzestan, Mollasani, Iran

4- Food and Nutritional Sciences Program, North Carolina Agricultural and Technical State University, E. Market Street, 1601, Greensboro, NC 24711, USA

5 -Department of Food Safety and Quality Control, Research Institute of Food Science and Technology (RIFST), Mashhad, Iran

ARTICLE INFO

Article History:

Received: 2024/5/20
Accepted: 2024/6/26

Keywords:

Melittin peptide,
Antibiofilm,
Pathogenicity,
Scanning electron microscope.

DOI: **10.22034/FSCT.21.150.192.**

*Corresponding Author E-Mail:
tabatabai@um.ac.ir

ABSTRACT

Escherichia coli and *Staphylococcus aureus* are pathogens that have the ability to form biofilms and cause disease in food products. Due to the fact that the enterotoxins produced by these two pathogens remain in a wide range of temperature, pH and saline conditions, they cause severe infections in humans. Melittin is a natural peptide derived from bee venom that can show its antimicrobial and anti-biofilm potential through disrupting the membrane of bacterial cells. For this purpose, in this study, the antimicrobial effect of this peptide on Gram positive and negative bacteria was investigated and its minimum inhibitory concentration **(**MIC) was determined as 100 µg/mL and 300 µg/mL, respectively. Also, the scanning electron microscope images confirmed the antimicrobial effect of the peptide on these two bacteria. Peptide melittin caused wrinkling, deformation and creation of holes in the cell membrane of treated bacteria, compared to the control sample. On the other hand, the results of the biofilm inhibition test showed that the addition of the peptide at a concentration of 2MIC completely prevented the biofilm formation of *S. aureus* prevented, while this value was equal to 91.00 ± 2.82 % in *E. coli* bacteria. Also, the increase in peptide concentration caused an increase in the destruction of adult biofilms of both bacteria. On the other hand, this peptide decreased the invasion and adhesion of these two bacteria to HT-29 and Caco-2 cells by reducing the mobility of pathogens. Therefore, according to the obtained results, melittin peptide can be a suitable alternative to chemical disinfectants that are harmful to the environment.

[DOI: 10.22034/FSCT.21.150.192]

1- Introduction

As people's living standards have risen and health awareness has grown, there has been a greater emphasis on food safety. Specifically, food poisoning caused by microbial contamination is the most common type of food safety issue [1, 2]. About 40-80% of microorganisms can form biofilms, which are associated with approximately 60% of food poisoning outbreaks worldwide [3]. Biofilms are complex structures made up of microorganisms stuck together by a protective layer of their own production or Exopolysaccharide (EXP). They form when bacteria attach to surfaces, grow, and multiply, going through stages of adhesion, colonization, and multiplication [4]. To effectively eliminate biofilms formed by pathogenic bacteria, a range of methods can be employed, including the use of disinfectants, physical removal, agents that disrupt the biofilm, heat treatment, and radiation [5, 6]. However, researchers and consumers are drawn to the idea of using natural and safe antimicrobial compounds, which offer a more attractive alternative to traditional methods, providing a safer and more effective way to combat biofilms.

E. coli, and *S. aureus* are recognized as the major pathogens that have the ability to form biofilms in the food industry [7, 8]. *S. aureus*, a Gram-positive and catalase-positive bacteria that produces enterotoxin, is frequently identified as a prominent foodborne pathogen linked to food safety concerns. It exhibits the capability to thrive within a broad temperature range (7 °C to 48 °C), tolerate a wide range of pH levels (4.2–9.3), and withstand varying concentrations of sodium chloride (up to 15%) [9]. It leads to infections affecting the heart, lungs, and endocardium [10]. *E. coli* is a Gram-negative bacteria, lacks spores, and has a rod-shaped structure. It is commonly present as a normal part of the intestinal microbiota in both humans and animals [11]. While the majority of *E. coli* strains are not harmful, certain pathogenic strains can cause gastrointestinal illness and respiratory pneumonia [12]. In the food industry, there is a significant challenge to identify and eliminate pathogens, as well as prevent them from causing disease and forming biofilms, which can have serious consequences.

Recent research has been focused on creating agents that can effectively eliminate biofilms, and antimicrobial peptides (AMPs) are being hailed as a promising solution to this problem [13, 14]. AMPs are the body's initial defense against pathogens, acting as a shield against infection. These small protein fragments, typically composed of 12 to 50 amino acids, exhibit anti-fungal, anti-viral, and anti-bacterial properties, making them effective at reducing bacterial load and disrupting biofilms. Their ability to quickly bind to membranes allows them to rapidly combat pathogens and prevent infection [15]. Melittin is a small, naturally occurring peptide found in bee venom, composed of 26 amino acids. Its potent antimicrobial properties are mainly due to the hydrophobic and cationic amino acids present in specific regions of the molecule, which contribute to its ability to combat microbial growth [16]. This peptide has the capability to create holes in the cell membrane, which can cause cell destruction, leakage of cellular contents, and ultimately, cell death, even at very low concentrations, by disrupting the membrane's function [17].

Therefore, according to the introduction that was stated, the objective of this study is to explore the antimicrobial and anti-biofilm properties of the melittin peptide against two prevalent pathogens in the food industry (i.e. *E. coli*, and *S. aureus*). Specifically, it aims to develop an effective solution based on this natural bioactive peptide to combat pathogenicity, inhibit

[DOI: 10.22034/FSCT.21.150.192]

biofilm formation, and enhance the quality of food products. This research seeks to provide an alternative to conventional methods currently used in this regard, offering a more natural and effective approach to addressing these issues.

2- Materials and methods

2.1. Bacterial strains

In this research, two harmful bacterial strains, specifically *E. coli* ATCC 33150 and *S. aureus* ATCC 25923, sourced from the Microorganism Collection Center at Ferdowsi University of Mashhad, Iran, were utilized. These strains were preserved at -80 °C in a liquid culture with 15% (v/v) glycerol added as a cryoprotectant.

2.2. Preparation of peptide

In a distinct study, the melittin peptide was produced and inserted into HEK293 cells. Since the produced protein was labeled with a His-tag at its terminus, the recombinant melittin peptide was purified through a Ni-NTA column, a form of Metal Affinity Chromatography resin [18].

2.3. Determination of the minimum inhibitory concentration (MIC)

The MIC values for two foodborne bacterial strains, *E. coli* ATCC 33150 and *S. aureus* ATCC 25923, were determined using the broth microdilution method in a 96-well plate with three repetitions. To carry out the experiment, the bacteria were initially cultured in their respective broth medium for 24 h at 37 °C. Following this, the bacterial concentration was adjusted to match a 0.5 McFarland standard. Next, 100 µL of the adjusted bacterial solution was added to each well. Subsequently, varying concentrations of the melittin peptide, ranging from 2500 to 9.76 µg/mL, were introduced into the wells containing the bacteria. The samples were then incubated at 37 °C for 24 h. The positive control consisted of the culture medium and the pathogen strain, while the negative control contained only the uninoculated culture medium. After the incubation period, a colorimetric analysis using a 0.5% tetrazolium chloride solution was performed to determine the MIC. Approximately 3 µL of this solution was added to each well and further incubated for 15 min at 37 °C. The MIC value was then determined as the lowest concentration of melittin that effectively inhibited the visible growth of pathogens [19].

2.4. Scanning electron microscope (SEM)

SEM was employed to investigate the impact of melittin peptide on the structure of pathogenic bacteria. Bacterial cells in the exponential growth phase were exposed to a concentration of 2MIC of melittin peptide and maintained for 24 h at 37 °C. Untreated bacteria served as the control group. Following the incubation period, the bacterial suspensions were centrifuged to form a pellet. This pellet was then rinsed twice with phosphate-buffered saline (PBS; pH 7.4) and subsequently fixed overnight in 4 mL of 2.5 % glutaraldehyde buffer. Subsequently, a series of ethanol solutions (20 %, 50 %, 80 %, and 100 %) at 4 °C for 10 min were used to dehydrate the bacterial cells. The samples were then coated with gold and examined using a scanning electron microscope (Zeiss (LEO) 1450 VP model, Germany) [20].

2.5. Quantification of biofilm formation and destruction

To assess the impact of melittin peptide on preventing biofilm formation by harmful bacteria, the method described by Rouhi et al. (2024) was followed. The bacteria were adjusted to a density of 10^6 CFU/mL. 50 µL of both bacterial cultures and antimicrobial peptides (2 MIC to 1/8 MIC) were placed in a 96-well polystyrene microtiter plate and kept in a 37 °C incubator for 72 h (50 µL fresh culture medium was added daily to each well to provide necessary nutrients for

[DOI: 10.22034/FSCT.21.150.192]

the bacteria). After the incubation period and biofilm development, the culture medium was removed, and each well was rinsed twice with 150 µL of PBS. The samples were dried in the incubator for 20 min. Subsequently, the amount of biofilm formed was measured using the colorimetric method with 150 µL of 0.1 crystal violet solution, and the plate was left in the environment for 30 min. The samples were rinsed thrice with distilled water, and the stained biofilm was exposed to 150 µL of a 98% ethanol solution. The optical density of each sample was assessed at OD570 nm utilizing a microplate reader (Model ELx808; Bio Tek, USA). The control group received only the broth culture medium, and the entire experiment was conducted three times for accuracy.

To assess the impact of melittin peptide on established bacterial suspansion biofilms, 100 µL of bacteria were added to each well and incubated for 72 h. Following this, the culture medium was removed, and the plate was washed. Then, each well was exposed to 2 MIC to 1/8 MIC of melittin peptide for 24 h. After the incubation period, washing and colorimetric procedures were carried out as outlined in biofilm inhibition method [21]. The inhibition and degradation rates were determined using the following formula:

$$\text{Inhibition and degradation ratio (\%)} = \frac{(C-B)-(T-B)}{C-B} \times 100$$

B = $OD_{570\ nm}$ of the negative controls, C = $OD_{570\ nm}$ of the control wells, and T = $OD_{570\ nm}$ of the treated wells.

2.6. Adhesion and invasion assay

The impact of melittin peptide on pathogenic bacteria's ability to invade and attach to host cells was studied using HT-29 cells (a human intestinal-epithelial cell line) and Caco-2 cells (a human colon adenocarcinoma cell line) [22].

To conduct the experiment, HT-29 and Caco-2 cells were seeded at a density of 5×10^5 cells per milliliter in a 24-well plate. The cells were then incubated for 18 h in an environment with 5% CO2 at a temperature of 37 °C. This incubation period allowed the cells to grow and reach full confluence, meaning they covered the entire surface area of the wells. Bacteria were grown in the presence and absence of MIC, 1/2 MIC, and 1/4 MIC concentrations of melittin until they reached the midpoint of their growth phase. The bacterial cells were then harvested, washed, and resuspended in DMEM to achieve a final concentration of 10^6 CFU/mL. HT-29 and Caco-2 cells were washed twice before the bacterial suspension was added to each well. The plates were then incubated in a humidified environment at 37 °C with 5% CO_2 for a period of 2 h.

To measure bacterial adhesion, cells with attached bacteria were first centrifuged at 600 × g for 5 min and then washed and lysed with incubation 1 mL of 0.1% (v/v) Triton X-100 at 4 °C for 20 min. The number of viable adherent bacteria was determined by performing serial dilutions and plating on TSA agar plates. The plates were then incubated at 37 °C for 24 h before the bacterial colonies were counted.

For invasion assays, the cell monolayers were incubated for 2 h after inoculation. They were then rinsed three times with PBS and incubated for an additional 30 min after adding DMEM supplemented with gentamicin (100 µg/mL) to eliminate extracellular bacteria. Subsequently, the cells were washed three times with PBS, lysed, and plated as described in the adhesion assay.

Adhesion and invasive rates were expressed as the percentage of the number of bacteria in the treatment groups relative to that of the control group.

2.7.Statistical analysis

[DOI: 10.22034/FSCT.21.150.192]

The experiments were conducted three times separately. The findings were presented as the average value plus or minus the standard deviation. Statistical analysis was performed using one-way analysis of variance, and the significance levels were determined using Duncan's multiple range test (DMRT) with the software SPSS version 25.0.

3-Results and Discussion

3.1. MIC

Table 1 displays the MIC values of melittin peptide against two strains, *E. coli*, and *S. aureus*. Various sub-MICs (1/2, 1/4, 1/8) of the peptide were chosen to assess its effects. Based on the findings, the melittin peptide exhibited significant antibacterial efficacy against all two strains of bacterial pathogens. However, to effectively inhibit the growth of *E. coli*, a Gram-negative bacteria with a distinct cell wall structure compared to the Gram-positive pathogens, a higher concentration of the peptide was required. The results suggest that Gram-positive bacteria are more susceptible to the antimicrobial peptide than Gram-negative bacteria. This is because Gram-negative bacteria have another layer called lipopolysaccharide in addition to the peptidoglycan layer, which serves as a protective barrier against antimicrobial agents, making it harder for them to penetrate the membrane and exert their effects. As a result, Gram-negative bacteria are more resistant to antimicrobial agents [23]. According to reports, the antimicrobial activity of AMPs is influenced by the composition of the cell envelope, which varies among different types of microorganisms. This variability explains why the peptide's effectiveness against bacteria can vary [24]. Cationic antimicrobial peptides, like melittin, tend to bind to negatively charged molecules found in both Gram-negative and Gram-positive bacteria. These molecules include lipopolysaccharides and teichoic and lipoteichoic acids. By binding to these molecules, the peptides are able to interact with the bacterial membrane and ultimately exert their antimicrobial effects [25]. AMPs function by disturbing the bacterial cell membrane and forming pores. This action facilitates the efflux of potassium ions, ultimately causing an imbalance in the electrochemical state across the membrane. Consequently, the bacterial cell is destroyed and dies. Moreover, AMPs also facilitate the passage of other substances, including antibiotics, into the cell, thereby augmenting their antimicrobial properties [26]. Our findings are in line with those of another study that examined the antimicrobial properties of vancomycin derivatives against *E. coli* and *S. aureus* strains that are resistant to vancomycin. The study revealed that Gram-negative bacteria exhibited higher MIC levels compared to Gram-positive bacteria [27]. The antimicrobial effect of thanatin was tested against Gram-positive bacteria, specifically *S. aureus*. The MIC for thanatin was found to be 2.57 µg/mL, which is significantly lower than its MIC against gram-negative bacteria, including *Shigella dysentery*, *E. coli*, *Salmonella typhi*, and *Salmonella paratyphi C*. For these Gram-negative bacteria, the MIC values ranged from 4.80 µg/mL to 76.92 µg/mL [28]. A recent study explored the impact of melittin peptide on Gram-negative and Gram-positive bacteria. The findings revealed that *E. coli* and *Pseudomonas aeruginosa*, both Gram-negative bacteria, exhibited significantly higher MIC levels (40-42.5 µg/mL and 65-70 µg/mL, respectively) compared to *S. aureus*, a Gram-positive bacterium, with a relatively low MIC range of 6-7 µg/mL [29].

Table 1. MIC of melittin peptide against bacteria *E. coli*, and *S. aureus*

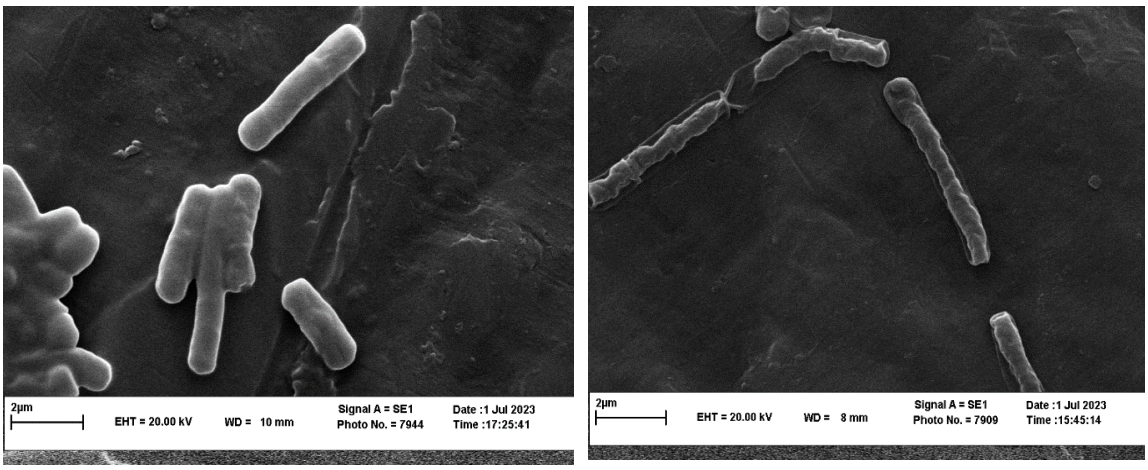
Bacterial species	MIC (µg/mL)
E. coli	300
S. aureus	100

[DOI: 10.22034/FSCT.21.150.192]

3.2. SEM

The SEM images revealed that normal cells in the control group had a smooth surface and well-defined outline, as seen in Fig 1A and 1C. In contrast, significant changes in cell morphology were observed when cells were treated with melittin, as shown in Fig 1B and 1D. The transformation in cell shape was accompanied by contraction, wrinkling, and damage to the bacterial cell wall, providing evidence that melittin's antibacterial activity is achieved by disrupting the bacterial membrane. Melittin's activity causes the cell membrane to break down, creating an imbalance in the cell's internal pressure and ultimately leading to the cell's death. Specifically, melittin's alignment with the membrane allows it to enter the membrane, stick together with other peptides, and distort the membrane's lipid structure, leading to the release of cellular contents [30]. Researchers have studied the impact of an antimicrobial peptide called AP138L-arg26 on the shape and structure of *S. aureus* bacteria. The results showed that the peptide disturbed the integrity of the cell membrane by more than 50%, and also led to a substantial increase in the release of potassium ions, which is detrimental to the bacterial membrane's integrity [31]. The research findings are consistent with observations made from SEM images, which examined the effects of thanatin on the cell structure of *E. coli*. The images demonstrate that as the peptide concentration increased, the cells contracted, became more indented and wrinkled, ultimately resulting in the bacteria's demise [32]. Another study [33] explored the effects of the antimicrobial peptide OaBac5mini on *E. coli* using similar imaging techniques. The treated bacteria exhibited signs of atrophy, corrugations, and the formation of pores on their cell membranes, accompanied by the leakage of intracellular contents. These observations provide insights into the effects at the cellular level.

(A) **(B)**



[DOI: 10.22034/FSCT.21.150.192]

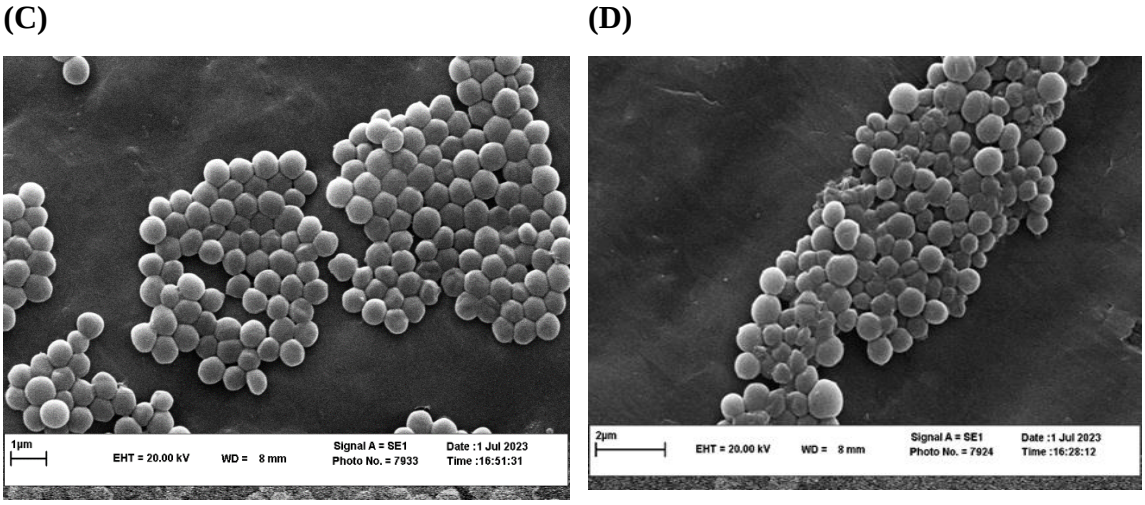


Fig. 1. SEM images of *E. coli* ATCC 33150 (**A**, untreated; **B**, treated with melittin), and *S. aureus* ATCC 25923 cells (**C**, untreated; **D**, treated with melittin).

3.3. Quantification of biofilm formation and destruction

The data in Table 2 highlights the impact of melittin peptide on biofilm formation in both *E. coli* and *S. aureus*, demonstrating its ability to inhibit this process.

Gram-positive bacteria exhibited a higher susceptibility to melittin peptide compared to Gram-negative bacteria. In Gram-positive bacteria, the formation of biofilms was completely inhibited at a concentration of 2 MIC, while at the MIC concentration, biofilm formation was inhibited by approximately 74.71±0.18 % in *S. aureus*. Conversely, *E. coli* demonstrated a lower inhibitory rate compared to the other bacterial strain. At the 2 MIC and MIC concentrations, it was able to prevent biofilm formation by approximately 91.00±2.82 % and 63.49±1.60 %, respectively. These results indicate that as the peptide concentration decreases, its inhibitory percentage also decreases. Overall, melittin peptide significantly prevented the formation of biofilms in the treated bacteria at different concentrations ($P < 0.05$).

Table 2. Biofilm inhibitory percentage of melittin peptide against *E. coli*, and *S. aureus*

Bacterial species	2 MIC (%)	MIC (%)	1/2 MIC (%)	1/4 MIC (%)	1/8 MIC (%)
E. coli	91.00±2.82	63.49±1.60	45.22±0.71	26.00±1.08	**1.90±0.75**
S. aureus	100	74.71±0.18	52.05±0.62	33.02±0.58	**6.15±0.09**

The process of eliminating mature biofilms involves the destruction of these complex structures. Notably, the melittin peptide showed significant effectiveness ($P < 0.05$) in breaking down well-established biofilms at various concentrations, including the MIC. Table 3 shows the exact percentage of destruction of pre-formed biofilms.

The highest effect of melittin peptide was observed on *S. aureus* at concentrations of 2 MIC and MIC, resulting in destructive percentages of approximately 86.75±1.77 % and 69.74±1.19 %, respectively. Conversely, the lowest effect was observed on *E. coli* at a concentration of 1/8 MIC with a destructive percentage of 0.27±0.85

[DOI: 10.22034/FSCT.21.150.192]

%, which was deemed insignificant. It is crucial to acknowledge that there are several factors that can influence the effectiveness of AMPs in preventing biofilm formation. These factors include the type of AMP being used, the dosage and duration of treatment, the difference in cell wall type of Gram-negative and Gram-positive bacteria as well as the specific pathogen that is being targeted [34].

The peptide 1018-K6 has been shown to have a significant ability to prevent *S. aureus* from forming biofilms. In fact, a very small amount of this peptide (80 μM) was enough to completely stop all strains of *S. aureus* from forming biofilms [35]. The supernatant and the breakdown of *Saccharomyces cerevisiae*, which has antibacterial properties, have been shown to effectively prevent the formation of biofilms by *S. aureus*. The supernatant reduced biofilm formation by 48%, while the breakdown of the bacteria reduced it by 69%, demonstrating its ability to inhibit biofilm formation [36]. The combination of LL-37 and PMB peptides was found to have a stronger anti-biofilm effect against *E. coli* and *P. aeruginosa* planktonic cells than when used individually. When both peptides were used together, the inhibition of biofilm formation and the destruction of pre-formed biofilms was significantly increased compared to when each peptide was used alone [37]. The results of this investigation are consistent with the research conducted by Picoli et al. (2017). They demonstrated a higher susceptibility of Gram-positive bacteria to melittin peptide in comparison to Gram-negative bacteria, which can be attributed to their distinct cell wall structures. Melittin peptide can readily penetrate the peptidoglycan layer of Gram-positive bacteria cell membranes, whereas creating pores in the lipopolysaccharide membrane of Gram-negative bacteria proves to be more challenging. The peptides facilitate the permeability of the cytoplasmic membrane and induce cell death by forming ion channels within the bacterial membrane.

Table 3. Biofilm destruction percentage of *E. coli*, and *S. aureus* treated with melittin peptide

Bacterial species	2 MIC (%)	MIC (%)	1/2 MIC (%)	1/4 MIC (%)	1/8 MIC (%)
E. coli	72.85±0.71	58.26±0.72	43.68±1.42	17.48±0.58	**0.27±0.85**
S. aureus	86.75±1.77	69.74±1.19	49.8±0.63	20.08±1.02	**4.58±0.60**

3.4. Adhesion and invasion assay

The ability of bacteria to adhere to surfaces is a crucial step in their colonization and ability to cause disease. This adhesive process is closely linked to the presence of carbohydrate-rich molecules on the bacterial cell wall, which play a key role in the bacteria's ability to attach and establish themselves [38]. Bacteria use their pili and flagella to swim and anchor themselves to host cells, allowing them to initiate the process of disease development and harm the host [39].

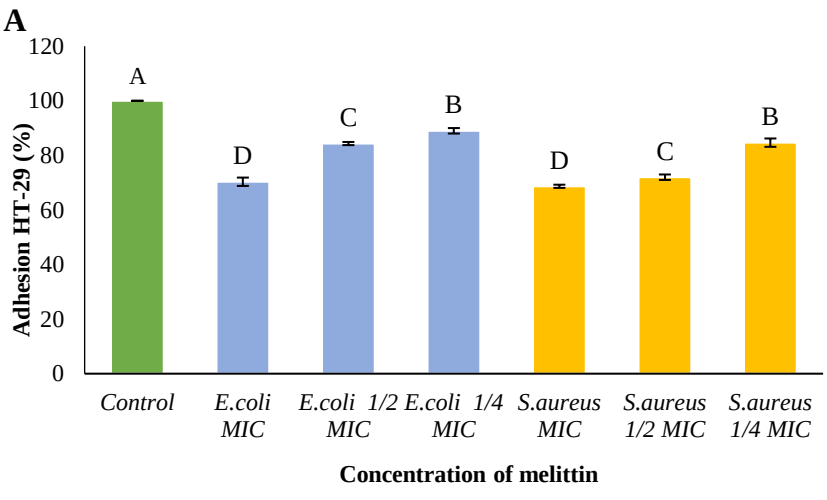
Our study reveals that the melittin peptide reduces the ability of *E. coli* and *S. aureus* to stick to intestinal cells (HT-29 and Caco-2) in a dose-dependent manner. As the dose of the peptide increases, bacterial adhesion decreases significantly at certain concentrations (Fig. 2A and 2B). The bacterial adhesion rates for *E. coli* and *S.*

[DOI: 10.22034/FSCT.21.150.192]

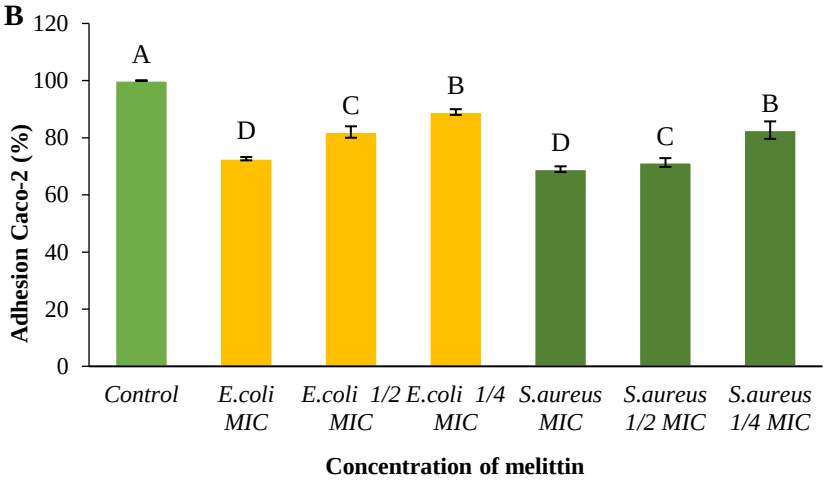
aureus when exposed to the MIC concentration, with 70.33 % and 68.66 % attachment to HT-29 cells, respectively. A similar trend was observed with Caco-2 cells, with adhesion rates of 72.66 % and 69.00 %. Compared to the control group, these adhesion percentages were significantly reduced ($P < 0.05$), indicating a notable decrease in bacterial attachment.

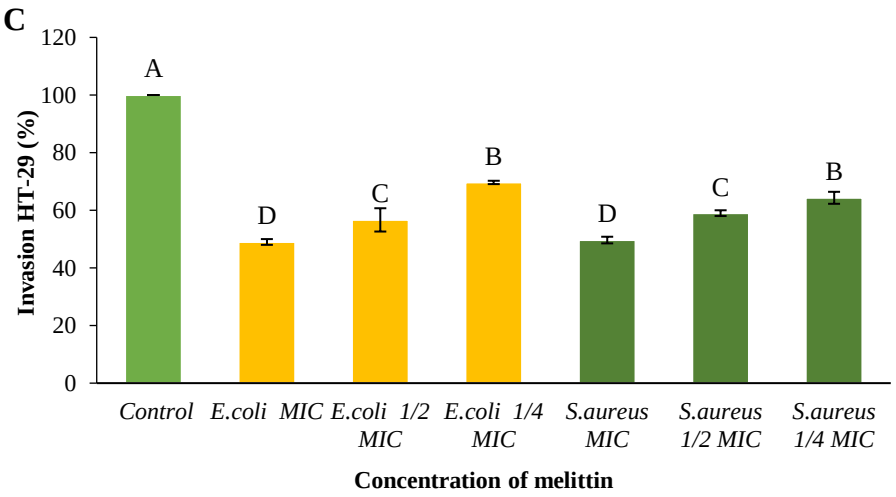
The Fig. 2C and 2D shows that melittin peptide significantly reduced the invasion of bacteria into HT-29 and Caco-2 cells. In comparison to the control group, the invasion of treated bacteria was significantly lower ($P < 0.05$). At the MIC concentration, melittin peptide inhibited the invasion of *E. coli* and *S. aureus* by 49.00 % and 49.66 % into HT-29 cells, and by 45.33 % and 48.00 % into Caco-2 cells. The study suggests that melittin peptide has the ability to prevent infection and disease development by blocking the adhesion and invasion of pathogenic bacteria, thereby highlighting its potential as a therapeutic agent.

Treatment of *E. coli* with plantarum strain L15 reduced the adhesion rate by 56%. This reduction can be attributed to factors such as competition for nutrients and receptors, as well as the production of antimicrobial compounds such as hydrogen peroxide, bacteriocins, organic acids and polysaccharides [40]. Another study found that the combination of trans-cinnamaldehyde and thymol was effective in preventing the adhesion of *L. monocytogenes* (a type of gram-positive bacteria) to Caco-2 cells in a way that was dependent [41]. A study found that a protein called PgTeL, derived from pomegranate, has the ability to prevent certain bacteria (including *Aeromonas*, *Salmonella*, *Serratia*, and *S. aureus*) from attaching to and invading human cells [42]. Our findings are consistent with a previous study that showed that the cell-free supernatant of *Lactiplantibacillus plantarum* can prevent *L. monocytogenes* from attaching to and penetrating human cells (Caco-2 and HT-29) at certain concentrations. This suggests that the bioactive compounds in the supernatant such as peptides have antimicrobial properties [43].



[DOI: 10.22034/FSCT.21.150.192]





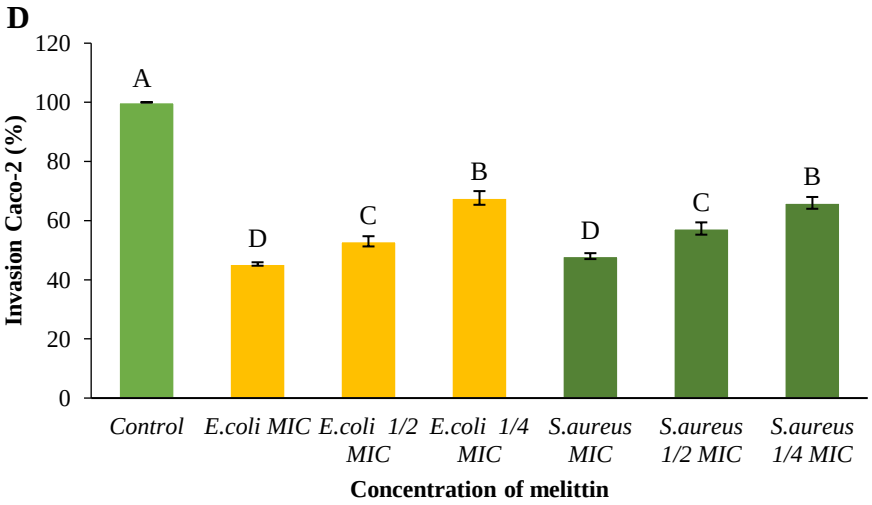


Fig. 2. Effects of the melittin peptide on adhesion to HT-29 cells (a), adhesion to Caco-2 cells (b), invasion to HT-29 cells (c), and invasion to Caco-2 cells (d) in *E. coli* ATCC 33150, and *S. aureus* ATCC 25923.

[DOI: 10.22034/FSCT.21.150.192]

4-Conclusion

Researchers have been actively seeking ways to combat biofilm formation and pathogenicity, particularly in the context of foodborne pathogens. Two common pathogenes, *E. coli* and *S. aureus*, have caused significant problems in the food industry by forming biofilms. In recent study found that melittin, a peptide derived from bee venom, has potent antimicrobial and anti-biofilm properties. The study's results suggest that melittin could be used as an alternative agent to prevent biofilm formation in food processing industries. Melittin was able to prevent biofilm formation by reducing cell adhesion and invasion, and preventing the spread of pathogens. The antimicrobial effect of melittin was confirmed through scanning electron microscopy. Overall, melittin exhibits strong antibacterial and anti-biofilm activity against food pathogens, making it a promising candidate for use as a disinfectant in the food industry.

5- Acknowledgements

This work has been supported by the Center for International Scientific Studies & Collaborations (CISSC), Ministry of Science Research and Technology of Iran (Funding number #4010372).

6-References

[1] Liu Y, Bu Y, Li J, Liu Y, Liu A, Gong P, et al. Inhibition activity of plantaricin Q7 produced by Lactobacillus plantarum Q7 against Listeria monocytogenes and its biofilm. Fermentation. 2022;8:75.

[2] Vasiee A, Falah F, Mortazavi SA. Evaluation of probiotic potential of autochthonous lactobacilli strains isolated from Zabuli yellow kashk, an Iranian dairy product. Journal of Applied Microbiology. 2022;133:3201-14.

[3] Galié S, García-Gutiérrez C, Miguélez EM, Villar CJ, Lombó F. Biofilms in the food industry: health aspects and control methods. Frontiers in microbiology. 2018;9:315815.

[4] Oloketuyi SF, Khan F. Inhibition strategies of Listeria monocytogenes biofilms—Current knowledge and future outlooks. Journal of basic microbiology. 2017;57:728-43.

[5] Xiang Y-Z, Wu G, Zhang Y-P, Yang L-Y, Zhang Y-M, Zhao Z-S, et al. Inhibitory effect of a new bacteriocin RSQ04 purified from Lactococcus lactis on Listeria monocytogenes and its application on model food systems. LWT. 2022;164:113626.

[6] Behbahani BA, Noshad M, Vasiee A, Brück WM. Probiotic Bacillus strains inhibit growth, biofilm formation, and virulence gene expression of Listeria monocytogenes. LWT. 2024;191:115596.

[7] Sofos JN, Geornaras I. Overview of current meat hygiene and safety risks and summary of recent studies on biofilms, and control of Escherichia coli O157: H7 in nonintact, and Listeria monocytogenes in ready-to-eat, meat products. Meat science. 2010;86:2-14.

[8] Behbahani BA, Yazdi FT, Mortazavi A, Gholian MM, Zendeboodi F, Vasiee A. Antimicrobial effect of Carboxy Methyl Cellulose (CMC) containing aqueous and ethanolic Eucalyptus camaldulensis L. leaves extract against Streptococcus pyogenes, Pseudomonas aeruginosa and Staphylococcus epidermidis. Archives of Advances in Biosciences. 2014;5.

[9] Economou V, Tsitsos A, Theodoridis A, Ambrosiadis I, Arsenos G. Effects of chitosan coatings on controlling Listeria monocytogenes and methicillin-resistant Staphylococcus aureus in beef and mutton cuts. Applied Sciences. 2022;12:11345.

[10] Tong SY, Davis JS, Eichenberger E, Holland TL, Fowler Jr VG. Staphylococcus aureus infections: epidemiology, pathophysiology, clinical manifestations, and management. Clinical microbiology reviews. 2015;28:603-61.

[11] Ma A, Neumann N, Chui L. Phenotypic and genetic determination of biofilm formation in heat resistant Escherichia coli possessing the locus of heat resistance. Microorganisms. 2021;9:403.

[12] Ma Y, Ding S, Fei Y, Liu G, Jang H, Fang J. Antimicrobial activity of anthocyanins and catechins against foodborne pathogens Escherichia coli and Salmonella. Food Control. 2019;106:106712.

[13] Talapko J, Škrlec I. The principles, mechanisms, and benefits of unconventional agents in the treatment of biofilm infection. Pharmaceuticals. 2020;13:299.

[DOI: 10.22034/FSCT.21.150.192]

[14] Yazdi FT, Tanhaeian A, Azghandi M, Vasiee A, Behbahani BA, Mortazavi SA, et al. Heterologous expression of thrombocidin-1 in Pichia pastoris: evaluation of its antibacterial and antioxidant activity. Microbial pathogenesis. 2019;127:91-6.
[15] Pontes JTCd, Toledo Borges AB, Roque-Borda CA, Pavan FR. Antimicrobial peptides as an alternative for the eradication of bacterial biofilms of multi-drug resistant bacteria. Pharmaceutics. 2022;14:642.
[16] Zarghami V, Ghorbani M, Bagheri KP, Shokrgozar MA. Melittin antimicrobial peptide thin layer on bone implant chitosan-antibiotic coatings and their bactericidal properties. Materials Chemistry and Physics. 2021;263:124432.
[17] Memariani H, Memariani M. Melittin as a promising anti-protozoan peptide: current knowledge and future prospects. Amb Express. 2021;11:69.
[18] Rouhi A, Yousefi Y, Falah F, Azghandi M, Behbahani BA, Tabatabaei-Yazdi F, et al. Exploring the Potential of Melittin Peptide: Expression, Purification, Anti-Pathogenic Properties, and Promising Applications as a Bio-Preservative for Beef Slices. LWT. 2024:116083.
[19] Alebooye P, Falah F, Vasiee A, Yazdi FT, Mortazavi SA. Spent coffee grounds as a potential culture medium for γ-aminobutyric acid (GABA) production by Levilactobacillus brevis PML1. Lwt. 2023;189:115553.
[20] Ghazanfari N, Fallah S, Vasiee A, Yazdi FT. Optimization of fermentation culture medium containing food waste for l-glutamate production using native lactic acid bacteria and comparison with industrial strain. LWT. 2023;184:114871.
[21] Kim YJ, Yu HH, Song YJ, Park YJ, Lee N-K, Paik H-D. Anti-biofilm effect of the cell-free supernatant of probiotic Saccharomyces cerevisiae against Listeria monocytogenes. Food Control. 2021;121:107667.
[22] Shi C, Sun Y, Liu Z, Guo D, Sun H, Sun Z, et al. Inhibition of Cronobacter sakazakii virulence factors by citral. Scientific Reports. 2017;7:43243.
[23] Nevalainen TJ, Graham GG, Scott KF. Antibacterial actions of secreted phospholipases A2. Review. Biochimica et Biophysica Acta (BBA)-Molecular and Cell Biology of Lipids. 2008;1781:1-9.
[24] Amiri EO, Farmani J, Amiri ZR, Dehestani A, Mohseni M. Antimicrobial activity, environmental sensitivity, mechanism of action, and food application of αs165-181 peptide. International Journal of Food Microbiology. 2021;358:109403.
[25] Ahmad Nejhad A, Alizadeh Behbahani B, Hojjati M, Vasiee A, Mehrnia MA. Identification of phytochemical, antioxidant, anticancer and antimicrobial potential of Calotropis procera leaf aqueous extract. Scientific Reports. 2023;13:14716.
[26] Herranz C, Driessen AJ. Sec-mediated secretion of bacteriocin enterocin P by Lactococcus lactis. Applied and environmental microbiology. 2005;71:1959-63.
[27] Wu C-L, Hsueh J-Y, Yip B-S, Chih Y-H, Peng K-L, Cheng J-W. Antimicrobial peptides display strong synergy with vancomycin against vancomycin-resistant E. faecium, S. aureus, and wild-type E. coli. International Journal of Molecular Sciences. 2020;21:4578.
[28] Tanhaeian A, Azghandi M, Mousavi Z, Javadmanesh A. Expression of thanatin in HEK293 cells and investigation of its antibacterial effects on some human pathogens. Protein and Peptide Letters. 2020;27:41-7.
[29] Picoli T, Peter CM, Zani JL, Waller SB, Lopes MG, Boesche KN, et al. Melittin and its potential in the destruction and inhibition of the biofilm formation by Staphylococcus aureus, Escherichia coli and Pseudomonas aeruginosa isolated from bovine milk. Microbial Pathogenesis. 2017;112:57-62.
[30] Lamas A, Arteaga V, Regal P, Vázquez B, Miranda JM, Cepeda A, et al. Antimicrobial activity of five apitoxins from apis mellifera on two common foodborne pathogens. Antibiotics. 2020;9:367.
[31] Zhang K, Yang N, Teng D, Mao R, Hao Y, Wang J. Expression and characterization of the new antimicrobial peptide AP138L-arg26 anti Staphylococcus aureus. Applied Microbiology and Biotechnology. 2024;108:1-18.
[32] Xia X, Song S, Zhang S, Wang W, Zhou J, Fan B, et al. The synergy of thanatin and cathelicidin-BF-15a3 combats Escherichia coli O157: H7. International Journal of Food Microbiology. 2023;386:110018.
[33] Shen S, Sun Y, Ren F, Blair J, Siasat P, Fan S, et al. Characteristics of antimicrobial peptide OaBac5mini and its bactericidal mechanism against Escherichia coli. Frontiers in Veterinary Science. 2023;10:1123054.

[DOI: 10.22034/FSCT.21.150.192]

[34] Rouhi A, Azghandi M, Mortazavi SA, Tabatabaei-Yazdi F, Vasiee A. Exploring the Anti-Biofilm Activity and Suppression of Virulence Genes Expression by Thanatin in Listeria monocytogenes. LWT. 2024:116084.
[35] Colagiorgi A, Festa R, Di Ciccio PA, Gogliettino M, Balestrieri M, Palmieri G, et al. Rapid biofilm eradication of the antimicrobial peptide 1018-K6 against Staphylococcus aureus: A new potential tool to fight bacterial biofilms. Food Control. 2020;107:106815.
[36] Saidi N, Owlia P, Marashi SMA, Saderi H. Inhibitory effect of probiotic yeast Saccharomyces cerevisiae on biofilm formation and expression of α-hemolysin and enterotoxin A genes of Staphylococcus aureus. Iranian Journal of Microbiology. 2019;11:246.
[37] Ridyard KE, Elsawy M, Mattrasingh D, Klein D, Strehmel J, Beaulieu C, et al. Synergy between Human Peptide LL-37 and Polymyxin B against Planktonic and Biofilm Cells of Escherichia coli and Pseudomonas aeruginosa. Antibiotics. 2023;12:389.
[38] Islam B, Khan AU. Lectins: To combat infections. Protein purification. 2012;1:167-88.
[39] Pizarro-Cerda J, Cossart P. Listeria monocytogenes: cell biology of invasion and intracellular growth. Microbiology spectrum. 2018;6:6.. 05.
[40] Behbahani BA, Noshad M, Falah F. Inhibition of Escherichia coli adhesion to human intestinal Caco-2 cells by probiotic candidate Lactobacillus plantarum strain L15. Microbial pathogenesis. 2019;136:103677.
[41] Upadhyay A, Johny AK, Amalaradjou MAR, Baskaran SA, Kim KS, Venkitanarayanan K. Plant-derived antimicrobials reduce Listeria monocytogenes virulence factors in vitro, and down-regulate expression of virulence genes. International Journal of Food Microbiology. 2012;157:88-94.
[42] Silva PM, Napoleão TH, Silva LC, Fortes DT, Lima TA, Zingali RB, et al. The juicy sarcotesta of Punica granatum contains a lectin that affects growth, survival as well as adherence and invasive capacities of human pathogenic bacteria. Journal of functional foods. 2016;27:695-702.
[43] Rouhi A, Falah F, Azghandi M, Behbahani BA, Mortazavi SA, Tabatabaei-Yazdi F, et al. Investigating the effect of Lactiplantibacillus plantarum TW57-4 in preventing biofilm formation and expression of virulence genes in Listeria monocytogenes ATCC 19115. LWT. 2024;191:115669.

[DOI: 10.22034/FSCT.21.150.192]

مجله علوم و صنایع غذایی ایران

سایت مجله: www.fsct.modares.ac.ir

مقاله علمی_پژوهشی

بررسی پتانسیل ضد میکروبی و آنتی بیوفیلمی پپتید ملیتین علیه *اشریشیا کلی* و *استافیلوکوکوس اورئوس*

آرزو روحی[۱]، فرشته فلاح[۱]، مرجان ازغندی[۲]، بهروز علیزاده بهبهانی[۳]، فریده طباطبایی یزدی[۱*]، سلام ابراهیم[٤]، علیرضا وسیعی[٥]

۱- گروه علوم و مهندسی صنایع غذایی، دانشکده کشاورزی، دانشگاه فردوسی مشهد، مشهد، ایران

۲- گروه علوم دامی، دانشکده کشاورزی، دانشگاه فردوسی مشهد، مشهد، ایران

۳- گروه علوم و مهندسی صنایع غذایی، دانشکده علوم دامی و صنایع غذایی، دانشگاه علوم کشاورزی و منابع طبیعی خوزستان، ملاثانی، ایران

٤- دپارتمان علوم غذایی و تغذیه، دانشگاه دولتی کشاورزی و فنی کارولینای شمالی، آمریکا

٥- گروه ایمنی و کنترل کیفیت مواد غذایی، موسسه پژوهشی علوم و صنایع غذایی، مشهد، ایران

اطلاعات مقاله

تاریخ های مقاله :

تاریخ دریافت:

تاریخ پذیرش:

کلمات کلیدی:

پپتید ملیتین،

آنتی بیوفیلم،

بیماری زایی،

میکروسکوپ الکترونی روبشی.

DOI: 10.22034/FSCT.21.150.192.

* مسئول مکاتبات:

tabatabai@um.ac.ir

چکیده

اشریشیا کلی و *استافیلوکوکوس اورئوس* پاتوژن‌هایی هستند که توانایی تشکیل بیوفیلم و ایجاد بیماری در محصولات غذایی را دارند. با توجه به اینکه انتروتوکسین‌های تولید شده توسط این دو عامل بیماری‌زا در محدوده وسیعی از دما، pH و شرایط شور باقی می‌ماند، باعث ایجاد عفونت‌های شدید در انسان می‌شوند. ملیتین یک پپتید طبیعی مشتق شده از زهر زنبور عسل است که می‌تواند پتانسیل ضد میکروبی و ضد بیوفیلم خود را از طریق مختل کردن غشای سلول‌های باکتریایی نشان دهد. بدین منظور در این مطالعه اثر ضد میکروبی این پپتید بر روی باکتری‌های گرم مثبت و گرم منفی بررسی و حداقل غلظت مهاری آن (MIC) به ترتیب ۱۰۰ میکروگرم بر میلی‌لیتر و ۳۰۰ میکروگرم بر میلی‌لیتر تعیین شد. همچنین تصاویر میکروسکوپ الکترونی روبشی اثر ضد میکروبی پپتید بر روی این دو باکتری را تایید کرد. پپتید ملیتین باعث ایجاد چین و چروک، تغییر شکل و ایجاد سوراخ در غشای سلولی باکتری‌های تیمار شده در مقایسه با نمونه شاهد شد. از سوی دیگر، نتایج آزمون مهار بیوفیلم نشان داد که افزودن پپتید در غلظت MIC ۲ به طور کامل از تشکیل بیوفیلم *استافیلوکوکوس اورئوس* جلوگیری کرد، در حالی که این مقدار در باکتری *اشرشیا کلی* برابر با ۹۱ درصد بود. همچنین افزایش غلظت پپتید باعث افزایش تخریب بیوفیلم بالغ هر دو باکتری شد. از سوی دیگر، این پپتید با کاهش تحرک پاتوژن‌ها، تهاجم و چسبندگی این دو باکتری به سلول‌های HT-29 و Caco-2 را کاهش داد. بنابراین با توجه به نتایج به‌دست‌آمده، پپتید ملیتین می‌تواند جایگزین مناسبی برای ضدعفونی‌کننده‌های شیمیایی مضر برای محیط زیست باشد.

[DOI: 10.22034/FSCT.21.150.192]