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Research Article

Antimicrobial and Antibiofilm Effects of Melittin and Apamin Bee Venoms from *Apis mellifera* L. on ESKAPE Pathogens and Cytotoxic Effects on L929 Fibroblast Cells

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Abstract

Public health is facing a worldwide challenge due to the emergence of multiple antibiotic resistance in ESKAPE *(Enterococcus faecalis*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Escherichia coli*) infections. The natural antimicrobial nature of bee venoms makes them promising antimicrobial candidates against antibiotic-resistant pathogens. In this study, melittin and apamin bee venoms were investigated to generate novel antimicrobial agents effective against ESKAPE microorganisms. The antimicrobial and antibiofilm activities of melittin and apamin synthesized from endemic *Apis mellifera* L. bee venom were tested against ESKAPE microorganisms using minimal inhibition, minimal bactericidal inhibition and time-kill curve methods. The in-vitro cytotoxicity effect of melittin and apamin for L929 fibroblast cells was also examined. Synthesized melittin and apamin (0.78-600 µg/mL) were antimicrobial against ESKAPE pathogens. Time-kill curve studies confirmed the growth retardation effect and bactericidal activity of melittin and apamin. Antibiofilm studies also showed that melittin and apamin significantly (melittin 8%-82%, apamin 1.8%-78%) inhibited the biofilm formed by ESKAPE pathogens. Melittin and apamin were not cytotoxic to L929 fibroblast cells. We found that melittin and apamin have high antibacterial properties. These naturally synthesized bee venoms offer a promising powerful solution to fight various pathogens.

Keywords: ESKAPE, Bee venom, Melittin, Apamin, Antimicrobial, Antibiofilm, Time-kill curve

Introduction

The growing effect that antimicrobial resistance (AMR) is having on morbidity, mortality, and healthcare expenditures makes it one of the most alarming public health issues in the world. AMR is linked to nearly 700.000 fatalities annually [1,2]. Antibiotic-resistant bacteria are predicted to be responsible for over 33.000 fatalities in the European Union (EU) by the year 2050 [3]. In spite of efforts to combat antimicrobial resistance (AMR), the overuse of antibiotics, particularly in developing nations where this practice permits the issuance of unnecessary prescriptions and the distribution of antibiotics to uninvited patients, the ongoing use of antibiotics, self-medication without consulting a doctor, and contact with nosocomial infections have resulted in the emergence of multidrug-resistant (MDR) bacteria. These bacteria account for approximately fifteen and a half percent of hospital-acquired infections (HAI) worldwide [4-7]. Among these pathogens, six species were considered particularly threatening due to their potential mechanisms and pathogenicity. ***E**nterococcus faecalis*, ***S**taphylococcus aureus*, ***K**lebsiella pneumoniae*, ***A**cinetobacter baumannii*, ***P**seudomonas aeruginosa*, and ***E**scherichia coli* are together referred to as the ESKAPE pathogens [8]. ESKAPE pathogens, which contribute significantly to the disease burden of countries and are associated with life-threatening bacteraemia, urinary tract infections, pneumonia, meningitis and wound infections, especially

in intensive care units, and are frequently isolated from clinical settings, are one of the leading causes of mortality and morbidity worldwide [9].

Antimicrobial peptides (AMPs) are cationic amphipathic peptides that are naturally occurring antibacterial compounds that are employed as one of the preferred antimicrobial groups in the therapy of infectious disorders due to the issue of antibiotic resistance. It has been demonstrated that AMPs' potential potency, quick action, and ability to eradicate both Gram-positive and Gram-negative bacteria have drawn interest in them as supplemental or alternative antibiotics [10]. Melittin is an alpha-helical peptide that belongs to the class of amphipathic polar molecules (AMPs). Its chemical formula is C131H228N38O32. The venom of *Apis mellifera*, the species of European honey bees, contains a water-soluble poisonous peptide called melettin, which makes about 52% of the dry weight of the venom [11]. This 26-amino acid peptide has a high surface activity. Its antimicrobial activity is thought to be due to its hydrophobic amino acids in the N-terminal and middle region (residues 1-20) and cationic amino acids in the C-terminal region (residues 21-24). Melittin is not only a potent antimicrobial but also a powerful anti-inflammatory and anticancer agent, and at low doses it increases cell growth and proliferation [12,13].

Apamin is a small peptide component derived from *Apis mellifera* [14]. Essential structure assurance appeared that apamin contains 18 amino corrosive buildups, with two intramolecular disulfide bonds (Cys1-Cys11 and Cys3-Cys15). In addition, the C-terminal buildup is amidated; this post-translational adjustment is common for peptide poisons from creature venoms [15]. Apamin has long been known as a particularly specific blocker of little conductance Ca^{2+}-activated K^+ (SK) channels [16], subsequently, it acts as an allosteric inhibitor [17]. Apamin is known to have antimicrobial activity among its pharmacological effect [18-20]. The aim of this study was to investigate the antibacterial and antibiofilm activity of melittin and apamin components from *Apis mellifera* L. on ESKAPE pathogens, important hospital pathogens, and their cytotoxic effect on L929 fibroblast cells.

Material and Methods

Ethical Statement

Ethics committee approval is not required for this study.

Isolation of Melittin and Apamin from *Apis mellifera* L. Bee Benom

Melittin and apamin used in the study were obtained from Çetinkaya [21]. Çetinkaya [21] isolated melittin and apamin from *Apis mellifera* L. bees in the bee pasture in Kütahya Dumlupınar University Evliya Çelebi Campus.

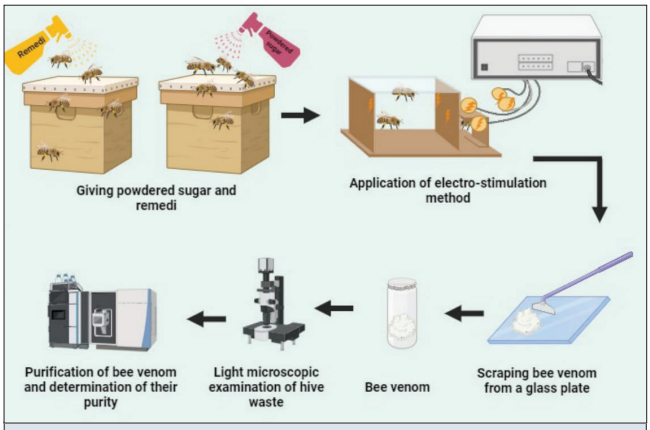

Fig 1. Bee venom collection scheme and process flow. CFU: colony-forming units

They sprayed using remediation, which encourages bees to avoid chemical-synthetic pesticides and prefer organic farming methods. They collected bee venoms by electro-stimulation as shown in *Fig. 1*, and measured their purity by reverse phase high pressure liquid chromatography (RP-HPLC) [21,22].

Antimicrobial and Antibiofilm Activity of Melittin and Apamin Components on ESKAPE Pathogens

- Bacterial Strains

E. faecalis American Type Culture Collection (ATCC) 29212, *S. aureus* ATCC 29213, *K. pneumoniae* ATCC 700603, *A. baumannii* ATCC 19606, *P. aeruginosa* ATCC 27853, and *E. coli* ATCC 25922 strains were used in this study. These standard strains stored at -80°C were inoculated on tryptic soy agar (TSA, Neogen®, Lansing, MI, USA) and incubated overnight at 37°C in an incubator. After incubation, bacterial suspensions with 0.5 McFarland turbidity were prepared [24].

- Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Determination

To determine the MIC values of antimicrobial agents by liquid microdilution method, 100 µL of cation added Mueller Hinton broth (MHB, Merck, Darmstadt, Germany) was added to all wells of 96 microplates. Melittin and apamin (10 mg/mL) were added 50 µL each to the first well and serial dilutions were made in duplicate (500-0.98 µg/mL). Finally, 50 µL of bacterial suspensions at 0.5 McFarland turbidity were added to each of these wells. To control bacterial growth, only bacteria (positive control) was added to one last row of the microplate and only MHB (negative control) was added to one well. The microplate was incubated in an incubator at 37°C for 18-20 h. The lowest concentration at which no growth was observed the next day was considered as the MIC value. MICs of antimicrobials were also determined. After the

MICs were determined, 100 µL samples were taken from each well that did not show growth and dripped onto TSA. The prepared petri dishes were incubated in an incubator at 37°C for 18-20 h. MBCs were defined as the concentration at which no growth was observed [25].

- Determination of Time Dependent Kill Curves (Kinetic Kill Curves)

Time-dependent killing level was performed to show the duration of action of the agents used. Melittin and apamin concentrations were prepared at 2X MIC values for all bacteria. Eppendorfs were filled with 500 µL of 0.5 McFarland turbid bacterial suspension and 500 µL of each concentration of melittin and apamin components. Incubated at 37°C. At 0 s, 1 h, 2 h, 4 h, 6 h and 24 h, 50 µL from each tube was added to 450 physiological saline (0.9%) and spread inoculated onto 100 µL TSA medium. After 24 h of incubation at 37°C, colonies formed on the petri dishes were counted and the number of bacteria per mL (cfu/mL) was calculated by taking into account the dilution factor. The time and concentration dependent change of bactericidal effect was taken as log10 value and this change was analyzed. Time-death graph was drawn according to the values obtained, time was shown on the "x" axis and the logarithmic value of the number of bacteria was shown on the "y" axis [26].

- Antibiofilm

The antibiofilm ability of melittin and apamin on ESKAPE pathogens was tested using the microtiter plate method. The first step involved growing single colonies overnight in 5 mL Tryptic Soya Broth (TSB)containing 1% glucose at 37°C with 180 rpm shaking. 100 µL of the bacterial suspension adjusted to 0.5 McFarland was added to 900 µL of TSB containing 1% glucose. 200 µL of this suspension was added to the wells of 96 U-shaped microplates and simultaneously serially diluted melittin and apamin in the range of 500 to 0.98 µg/mL in a volume of 200 µL. Incubated at 37°C for 1 night with 180 rpm in a shaking incubator. Following the removal of the wells' contents, the wells were air dried and cleaned three times using 200 µL of phosphate buffer saline (PBS) regular saline. Following the addition of 200 µL of 95% methanol to each well, the wells were aspirated after 15 min, dried once more at room temperature, and then the dye was removed after 5 min of staining with 0.1% crystal violet in a 200 µL volume. Wells were washed 3 times with PBS and air-dried again. Finally, 200 µL of 33% glacial acetic acid for Gram-positive bacteria and 95% ethanol for Gram-negative bacteria were added to the wells and the absorbance at 600 nm was recorded using a microplate reader after 15 min at room temperature [27]. The minimum biofilm eradicating concentration (MBEK) was determined as the concentration at which the absorbance was less than or equal to the negative control. The percentage of biofilm inhibition was calculated as follows [28]: MBIC = [1-(OD test/OD control)] × 100.

Effects of Melittin and Apamin Components on The Proliferation of L929 Fibroblast Cells

- L929 Fibroblast Cell Culture

The effects of melittin and apamin on healthy cells were determined using mouse connective tissue fibroblast cells (L929). L929 fibroblast (CCL-1) cells were obtained commercially from ATCC (Manassas, USA). Before starting the cell culture study, medium containing DMEM (Dulbecco's Modified Eagle's Medium - high glucose ATCC, USA) + 10% fetal bovine serum (FBS; ATCC, USA) + penicillin/streptomycin (100 µg/mL; Gibco, USA) was prepared under sterile conditions in a UV cabinet.

Cells were suspended in medium and cultured in 25 cm^2 and 75 cm^2 cell culture flasks under aseptic conditions in an incubator at 37°C, 95% humidity and 5%CO_2. During the cell culture periods, the adhesion of the cells to the surface, their potential to fill the flask surface and the density of the cells were examined under an inverted microscope (ZEISS Primovert, GERMANY).

- Application of Melittin and Apamin *to L929 Fibroblast Cells and Calculation of IC50 Dose with Rreal-Time Cell Analysis System*

Melittin and apamin were mechanically prepared in cell culture medium. The determination of the number of cells to be used in the analysis to determine the IC50 dose of melittin and apamin to L929 fibroblast cells propagated by cell culture studies was performed using the real-time cell analysis system xCELLigence™ (ACEA Biosciences, Inc., San Diego, CA, USA). To determine the IC50 value, 50000 cells per well were seeded on xCELLigence E-platters and apamin and melittin were added to the medium at decreasing concentrations (5 µM, 4 µM, 3 µM, 2 µM, 1 µM, 500 nM and 250 nM) and measurements were taken at 15 min intervals for 120 h. For control, only medium was added. IC_{50} value was analyzed simultaneously according to logarithmic increase in the xCELLigence system [29].

Statistical Analysis

To evaluate the cell culture cytotoxicity results obtained from the system, statistical analyses were carried out using the xCELLigence system software. The system software fits an optional "sigmoidal dose–response equation" curve to the experimental data points in order to calculate logarithmic half maximum inhibitory concentration (log [IC_{50}) values. Statistical analyses were performed with Prism version 9 (GraphPad Software, California, USA). The t-test was used to determine the differences between more than one independent group. All

Table 1. *Minimum inhibitory concentrations (MICs) and minimum bactericidal concentrations (MBCs) of melittin and apamin against ESKAPE bacteria*

Strains	MIC (µg/mL)		MBC (µg/mL)	
	Melittin	Apamin	Melittin	Apamin
E. faecalis ATCC 29212	500	500	500	500
S. aureus ATCC 29213	7.812	250	31.25	250
K. pneumoniae ATCC 700603	62.5	250	62.5	500
A. baumannii ATCC 19606	125	7.812	125	7.812
P. aeruginosa ATCC 27853	0.98	250	0.98	250
E. coli ATCC 25922	7.812	500	15.625	500

MIC: *minimum inhibitory concentration;* ***MBC:*** *minimum bactericidal concentration*

data are presented as mean ± SD. P<0.05 was considered statistically significant.

Results

Mass Analysis of Melittin and Apamin

LC-MS mass spectra were used to identify, characterize and quantify melittin and apamin compounds in bee venom, and these spectra are important for understanding their pharmacological effects and potential uses. The molecular mass of the apamin peptide (M): 2026.9037 Da, while the molecular mass of melittin peptide (M): 2845.7725 Da [21].

Antimicrobial Activities of Melittin and Apamin

- Results of Minimum Inhibitory Concentration (MIC) and Minimum Bactericidal Concentration (MBC) Values

The MIC and MBC activities of melittin and apamin on ESKAPE pathogens are given in *Table 1*. The MIC values of melittin on ESKAPE bacteria were between 500-0.98 µg/mL, while apamin was detected at concentrations of 500-7.812 µg/mL. MBC values were also similar to the MIC concentrations. The lowest MIC and MBC values were 0.98 µg/mL for melittin on *P. aeruginosa* ATCC 27853 and 7.812 µg/mL for apamin on *A. baumannii* ATCC 19606 strain *(Table 1).*

- Results for Time-Dependent Kill Curves

According to the analysis of time-dependent killing curves, melittin and apamin were found to be bactericidal against all ESKAPE pathogens. Melittin and apamin were 1st and 8th on *E. faecalis* ATCC 29212, *S. aureus* ATCC 29213, 1st and 12th on *K. pneumoniae* ATCC 700603, 12th and 8th on *A. baumannii* ATCC 19606, 2nd and 8th on *P. aeruginosa* ATCC 27853, and 12th and 1st on *E. coli* ATCC 25922, respectively *(Fig. 2).*

- Antibiofilm Activities of Melittin and Apamin

Biofilm inhibition activity of melittin and apamin was performed against ESKAPE isolates. As shown in *Table 2*, melittin and apamin inhibited biofilm for ESKAPE pathogens to varying degrees. The percentage of biofilm inhibition was higher for *E. faecalis, S. aureus, K. pneumoniae, A. baumannii* in the presence of melittin than apamin at the same concentrations. For *P. aeruginosa and E. coli,* the percentage of inhibition was higher in the presence of apamin. In all isolates, inhibition was observed in the presence of melittin and apamin at concentrations of 7.812 µg/mL and above. At concentrations of 3.9 µg/mL and below, melittin and apamin did not inhibit biofilm on *P. aeruginosa* (3.9 µg/mL), *K. pneumoniae* (1.95 µg/mL),

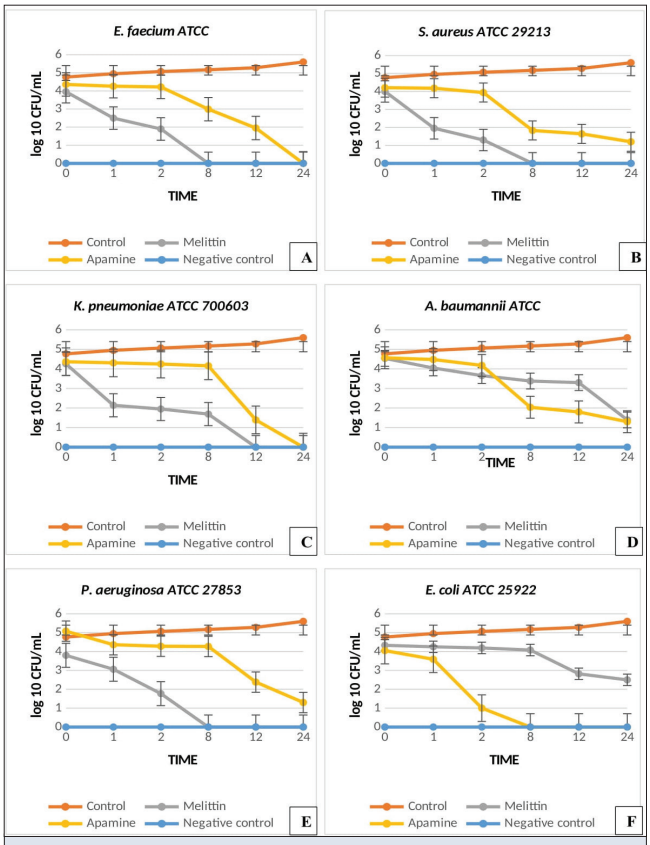

Fig 2. Time-kill assays of melittin and apamin for (A) *E. faecalis* ATCC 29212 (B) *S. aureus* ATCC 29213, (C) *K. pneumoniae* ATCC 700603, (D) *A. baumannii* ATCC 19606, (E) *P. aeruginosa* ATCC 27853, (F) *E. coli* ATCC 25922. X-axis represents killing time and Y-axis represents logarithmic (log) growth phase. Error bars indicate standard deviation

Table 2. *Percent inhibition (%) of biofilm formation of ESKAPE pathogens at different concentrations*

Microorganisms	Concentrations (µg/mL)																			
	500		250		125		62.5		31.25		15.625		7.812		3.9		1.95		0.98	
	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A	M	A
Enterococcus faecium	81.9	77.5	79.4	77.3	78.3	71.1	76.5	70.3	72.3	69.9	68.4	67.4	66.4	64.9	63.4	60.8	59.6	59.1	58.7	57.0
Staphylococcus areus	48.0	16.9	45.3	7.7	43.3	3.9	38.6	3.7	32.5	3.6	28.2	1.8	27.3	0.3	14.3	0	10.7	0	2.8	0
Klebsiella pneumoniae	59.6	51.9	49.5	48.4	41.7	37.7	32.2	37.1	18.9	35.6	16.7	33.2	8.4	32.3	4.8	31.0	0	30.5	0	8.8
Acinetobacter baumannii	59.6	53.3	59.5	40.4	51.4	37.0	48.1	30.5	40.6	21.4	40.0	19.1	43.5	21.5	32.1	10.0	25.8	7.6	27.5	5.2
Pseudomonas aeruginosa	57.6	62.7	53.7	60.4	53.3	57.6	43.1	58.2	39.9	57.6	28.6	56.4	8.5	53.9	0	53.0	0	52.9	0	50.0
Escherichia coli	62.0	68.9	56.8	48.5	54.1	46.7	46.6	46.3	43.3	46.0	42.0	46.4	41.8	45.3	33.8	44.0	25.1	43.6	11.8	38.3

M: melittin; A: apamin

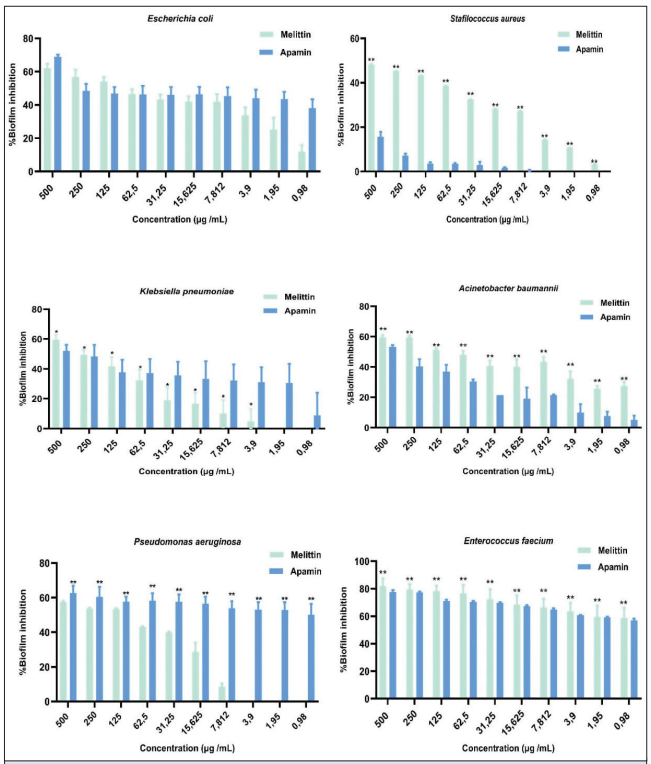

Fig 3. The graph shows the biofilm inhibition activity of melittin and apamin against ESKAPE pathogens. Error bars represent standard deviations. *P<0.05 and **P<0.001. Data were analyzed using Graphpad t-tests (non parametric test)

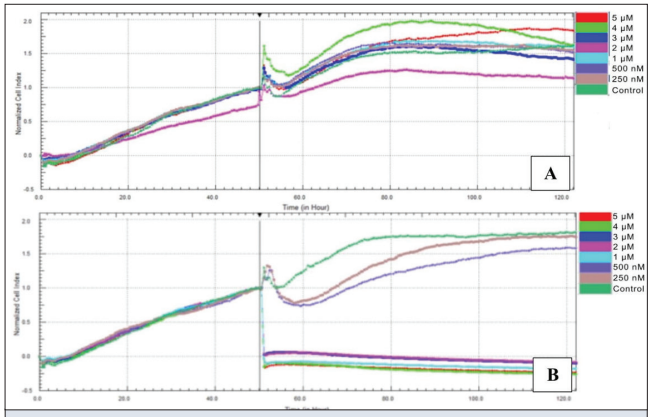

Fig 4. Time curves normalized cell index. Comparison of L929 cell proliferation after treatment with 5 µM, 4 µM, 3 µM, 2 µM, 1 µM, 500 nM and 250 nM (A): apamin and (B): mellitine. The arrow indicates the time of substance application

and *S. aureus* (3.9 µg/mL). Among the six pathogens, both melittin and apamin gave a high percentage of inhibition on *E. faecalis* isolate *(Fig. 3)*.

Effects of Melittin and Apamin Components on the Proliferation of L929 Fibroblast Cells

- Effects of Apamin on the Proliferation of L929 Cells

The software of the xCELLigence device automatically calculated IC_{50} values as 4.2 µM at 24 h, 4.9 µM at 48 h and 4.8 µM at 72 h after apamin treatment of L929 cells at different doses *(Fig. 4-A)*.

- Effects of Melittin on the Proliferation of L929 Cells

The software of the xCELLigence device automatically calculated IC_{50} values as 152 nM at 24 h, 87 nM at 48 h and 76 nM at 72 h after melittin treatment at different doses to L929 cells *(Fig. 4-B)*.

Discussion

The ability to effectively manage bacterial infections is threatened by rising rates of nosocomial infections and antibiotic resistance. Worldwide, the most common causes of nosocomial infections are ESKAPE pathogens, including *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Escherichia coli*. The majority of the

ESKAPE pathogens are isolated from hospital units' blood, urine, wound swabs, respiratory samples, etc. (intensive care units (ICU), medical and surgical units) [31]. Even though hospitals are the main source, those with weakened immune systems-such as those with diabetes, chronic renal disease, lung disease, and cancer patients-are the most susceptible [32]. ESKAPE pathogens, which fall into many resistance categories (low-resistance, multi-drug and very drug resistant, pan-resistant bacteria), are the most common bacteria with an antibiotic resistance profile used as a sentinel in monitoring systems [33]. The multidrug resistance shown by these pathogens is a worldwide burden and treatment of patients with conventional antibiotics is becoming increasingly difficult or ineffective [34]. Antibiotics are known to interfere with the formation of cell walls, proteins, RNA, DNA, membrane stability, and energy metabolism. By altering these targets, deactivating antibiotics, or expelling them from cells, bacteria might develop resistance [34]. Combinations of antimicrobial drugs targeting different molecular targets than antibiotics can help combat mechanisms of resistance to antibiotics, broaden their antibacterial spectrum, reduce their toxicity and increase their efficacy [35]. It has been shown in various studies that melittin and bee venoms have broad-spectrum antibacterial effects and antibiofilm properties [36-41]. In this study, we tested the safety profile of melittin and apamin bee venoms from *Apis mellifera* L. using antimicrobial, antibiofilm and cytotoxicity methods.

Studies have shown the antibacterial activity of melittin [36-40]. Han et al.[38] in their antimicrobial analysis with melittin, reported the MIC value for *E. coli* as 0.125 µg/mL and for *S. aureus* as 0.06 µg/mL. Again, Maitip et al.[42] found that MIC and MBK values were 266 µg/mL for *E. coli*, respectively; 400 µg/mL; 400 µg/mL, 400 µg/mL for *K. pneumonia*; They found it to be 41 µg/mL and 41 µg/mL for *S. aureus*. In another experimental study investigating the antimicrobial activity of melittin, MIC and MBK values were given for *K. pneumoniae* (100 µg/mL; 300 µg/mL), *E. coli* (30 µg/mL; 60 µg/mL), *P. aeruginosa* (100 µg/mL;100 µg/mL), *A. baumannii* (30 µg/mL; 30 µg/mL) and *E. faecium* (1 µg/mL; 4 µg/mL) [43,44]. In our study, melittin showed antimicrobial properties at higher concentrations for *E. faecium* and at lower concentrations for other pathogens, giving different results from the literature. Apamin bee venom obtained from *Apis mellifera* L. showed antimicrobial activity on *E. coli* [45]. However, Kuzmenkov et al.[20] found that apamin did not show antimicrobial effect for *E. faecalis, S. aureus, E. coli* and *P. aeruginosa* up to a very high concentration of 50 µM. In our study, both melittin and apamin showed antimicrobial effect for all pathogens. Melittin showed antimicrobial effect at lower concentrations for all pathogens except *A. baumannii*. Apamin, on the contrary, showed antimicrobial effect at lower concentrations in *A. baumannii* pathogen compared to other pathogens.

Time-dependent killing assay was performed to monitor cell viability against time. Dosler et al.[46] determined the time-dependent killing assay results of melittin as 2-7 h for *P. aeruginosa*, *E. coli* and *K. pneumoniae*. In another study, time-dependent killing analysis results for *A. baumannii* (at 3rd, 5th, and 24th h), *S. aureus* (at 5th, and 24th h), and *K. pneumonia* (at 3rd, 5th, and 24th h) showed bactericidal effect at different concentrations [47]. The results of the time-dependent killing assay in our study showed that the two antimicrobial peptides, especially melittin, were rapidly bactericidal against *E. faecium, P. aeruginosa, K. pneumoniae* and *S. aureus* strains in a concentration-dependent manner within 1-8 h, followed by a significant logarithmic decrease at 1 h and bactericidal at 8 h. For *E. coli* and *A. baumannii*, a significant logarithmic decrease was observed at 12 and 24 h, which is consistent with the literature. In a study investigating the antibacterial effect of apamin on methicillin-resistant *S. aureus*, vancomycin-resistant *E. faecalis*, carbapenem-resistant *E. coli*, carbapenem-resistant *K. pneumoniae* and carbapenem-resistant *Acinetobacter* species, it was found that the number of bacteria decreased by 4-6 logs within 1-24 h [48]. In our study, the times of logarithmic decrease and bactericidal properties were 8-24 h for *E. faecium, A. baumannii* and *S. aureus*, 12-24 h for *K. pneumoniae* and *P. aeruginosa* and 2-8 h for *E. coli*.

Medical science today faces the difficult problem of managing infections associated with biofilms. The antibiotic therapy used is typically insufficient to completely eradicate bacterial biofilms in this situation. This necessitates the use of large quantities of antimicrobial drugs, which are often equally ineffective, and sometimes even periodic prescriptions with a high risk of side effects and the emergence of new resistant strains [49]. It has been reported in different studies that melittin, an antimicrobial peptide, has strong antibacterial activity as well as strong antibiofilm activity [46,49-51]. Our study was consistent with the literature and showed a strong biofilm inhibition effect of melittin on ESKAPE pathogens with biofilm inhibition rates ranging from 82% to 8% in the concentration range of 500 to 7.812 µg/mL. To the best of our knowledge, there is no study investigating the antibiofilm activity of apamin. We determined the biofilm inhibition rate of apamin as 1.8% to 78% in the concentration range of 500-15.625 µg/mL.

While the antimicrobial effect of melittin and apamin is of importance, we investigated the survival rate of L929 fibroblasts. Zorilă et al.[52] reported a decrease of less than 50% in the viability of L929 cells up to 2.5 µM melittin and cell viability ranged from 55% to 63% for the highest

concentration tested. On the contrary, Klubthawee, et al.[53] reported that melittin was very cytotoxic against L929 cells and significantly reduced cell viability. In our study, it was found that the effective dose amount of melittin in L929 fibroblasts decreased with time. It was determined that melittin application above 500 nM had a lethal effect for L929 cells. In our study, similar to mellitine, apamin showed an effect at decreasing doses depending on time. However, apamin was not as effective as mellitine in L292 cells.

In conclusion, while antimicrobial resistance for ESKAPE pathogens is increasing and new antibiotics are not being developed to combat antibiotic-resistant microorganisms, melittin and apamin may be a promising alternative strategy against drug resistance of these microorganisms. In our study, we report the antimicrobial, antibiofilm and cytotoxic effects of melittin and apamin using ESKAPE ATCC strains. Melittin and apamin showed growth inhibition of ESKAPE microorganisms. Melittin and apamin also inhibited biofilm formation at extremely low concentrations. However, melittin and apamin synthesized in this study also had a positive effect on L929 cells. Our research thus indicates that melittin and apamin are potential antibacterial agents against strains of ESKAPE. To learn more about potential additional processes behind melittin and apamin's antibacterial action, more mechanistic research is being conducted. It is also necessary to look at the synergistic effects of melittin and apamin when used with antibiotics. The universality of the potential anticancer activity of these bee venoms also needs to be confirmed using cancer cell lines. This endemically synthesized melittin and apamin effective.

Declarations

Availability of Data and Materials: Materials and data sets from the study are available upon request from the corresponding author (E. Aydın).

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Ethical Approval: Ethics committee approval is not required for this study.

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

Author Contributions: All authors participated in designing the methodology, EA, MKG, AKS: writing the original manuscript, EA, SC, MKG, AKS: software and visualization, EA, SC, MKG, AKS: data curation, EA, SC, MKG, AKS: research and validation, EA, MKG, AKS: review and editing.

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